THE EFFECTS OF CERTAIN ANTIHYPERTENSIVE DRUGS ON THE TISSUE RESPIRATION OF NORMAL AND HYPERTENSIVE RATS

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ABSTRACT

An investigation was undertaken to determine the effects of reserpine, sodium azide and hydralazine on the blood pressure and tissue respiration of normal and hypertensive rats. QO_2 values were determined using samples of the same organ in Krebs Ringer Phosphate (KRP), in Krebs Medium III (KMIII) and suspended in oxygen (HM flasks). The drugs were administered to normal and DCA hypertensive rats for forty to fifty days prior to tissue respiration studies.

Hypertension was produced in rats by using the Grollman and Desoxycorticosterone (DCA) methods. When weanling rats were subjected to a short period of choline deficiency a substantially raised blood pressure was not produced. Reserpine, hydralazine and sodium azide caused an increased precent survival and a reduction in the mean blood pressure of the DCA implanted rats, but failed to reduce the pressure to the normal value. The drugs had little effect on the blood pressure of normotensive rats.

The respiration of heart and kidney slices from rats made hypertensive by the Grollman or DCA saline methods was lower than that of normal rats. Both reserpine and hydralazine tended to return to normal the decreased kidney respiration which was observed in the untreated hypertensive rats. Sodium azide caused an increase in the respiration rate of hypertensive kidney slices. The decreased respiration of the hypertensive heart was returned towards normal when the animals were treated with the antihypertensive drugs.

It is suggested that the Huston-Martin technique has several advantages over the conventional Warburg technique in pharmacologic investigations at the cellular level.

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I. INTRODUCTION

A group of related, chronic, degenerative conditions classified as cardiovascular renal diseases lead todays mortality list. One third the total number of deaths in Canada are its victims. Cardiovascular renal diseases can manifest themselves in a number of different symptoms and have a variety of causes. Fifty percent of all victims in this group suffer from essential hypertension. In this condition there is an increase in peripheral resistance of the vascular system due to constriction of the peripheral arterioles and as a compensatory mechanism the diastolic and systolic blood pressure becomes elevated. The cause of this vaso-constriction in the absence of recognizable structural disease is not known.

The lack of knowledge as to the basic etiology of essential hypertension has prevented the development of corrective therapy and at present treatment is directed toward the symptoms rather than the underlying causes of the disease. However, since it has been shown (1)(2) that effective reduction of blood pressure will arrest or retard the pathological processes associated with the disease, the adequate and prolonged lowering of the arterial pressure becomes the first and most urgent aim in treatment of hypertension. Many of the newer antihypertensive agents are said to fulfill these requirements. However, it is still the aim of researchers to produce better antihypertensive agents and ultimately to determine the etiology of the disease.

Considerable knowledge concerning essential hypertension has accumulated and many theories regarding the pathogenesis of this condition have been proposed. However none of these theories adequately explains all aspects of essential hypertension.

One of the foremost findings in this field is that by relatively simple techniques it is possible to produce hypertension in animals and thus the disease may be studied experimentally. By investigating at the cellular level we may obtain a better understanding of the actions of the antihypertensive agents and eventually learn more of the complex mechanism underlying hypertensive disease itself.

II. CHEMISTRY AND GENERAL PHARMACOLOGY OF DRUGS USED IN THE INVESTIGATION

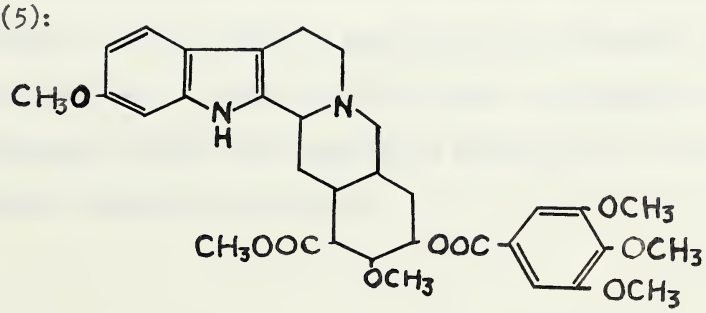
A knowledge of the chemistry and the general pharmacological activities of the antihypertensive drugs used is necessary for an understanding of the mechanism of action of the drugs on the biochemical phenomena being investigated and the interpretation of the observed effects.

1. Reserpine

Although extracts from Rauwolfia, a genus of the family Apocynaceae, have been used since ancient times for the treatment of a large number of diseases, it was not until 1930 that studies of the plant aroused the world wide interest of scientists and clinicians. Since that time a vast amount of research has dealt with the actions of the alkaloids derived from Rauwolfia. These investigations have been reviewed in detail by Lewis (3) and Bein (4).

Although the root of Rauwolfia serpentina contains numerous other substances which modify its actions, reserpine is probably the main active principle and for this reason it has been the alkaloid most studied.

Reserpine is a relatively weakly basic alkaloid isolated from an oleo resin fraction of Rauwolfia serpentina. It has a melting point of 262-263° C and presents a blue fluorescence in ultraviolet light. Reserpine has been recently synthesized (6) and has the following chemical structure (5):



Reserpine exhibits a complex pattern of activity, its primary site of action being on the central nervous system. It exerts secondary effects on certain other tissues. The several effects of reserpine vary in intensity from one species of animal to another but the drug has two features which are common to all: the onset of action occurs after a certain latent period and single doses have a long duration of effect.

On the central nervous system reserpine produces generalized depression which is not similar to that produced by barbiturates. It has no analgesic activity. Its action can be described as one of relaxation and tranquilization. The effects of reserpine on the cardiovascular system are thought to be due to direct action on the central mechanisms which regulate circulation, probably the hypothalamus. Evidence has also shown that reserpine produces a direct peripheral relaxant effect on blood vessels (7). Bradycardia occurs only after high doses of reserpine and is of central origin. For the most part reserpine produces a slowly developing fall in elevated blood pressure which is due to vasodilation and in unaccompanied by a reduction in cardiac performance. The higher the level of blood pressure prior to the administration of reserpine the greater will be the subsequent reduction. Usually in normotensive animals little effect is shown. Increasing doses of reserpine tend to prolong rather than intensify its effects on the blood pressure. Cardiac output, renal hemodynamics, renal water and electrolyte excretion are inconsistently altered (9).

There is little effect of the drug upon respiration unless very large doses are given, in which cases decreased respiratory rate may result. Reserpine lowers body temperature probably due to depression of the temperature regulating mechanism.

Other effects of reserpine which are thought to be due to its action on the central nervous system include miosis, increased intestinal peristalsis and secretion and relaxation of the nicitating membranes.

The alkaloid is absorbed from the gastro intestinal tract, rapidly removed from the blood stream and concentrated in the fatty tissue. Sixty percent of the alkaloid is excreted by the kidney in 3 to 4 hours after oral administration (7). Recently it has been postulated that the central effects of reserpine are mediated by the liberation of serotonin (64)(65)(66)(67)(68). More work must be done with regards to this theory before anything conclusive can be stated.

There are no serious side effects nor the developing of tolerance following administration of reserpine. Occasionally nasal congestion, diarrhea, nausea or anorexia develop. Hallucinations and mental depression have occasionally resulted with high doses of reserpine.

2. Sodium Azide

Studies on the toxicity of sodium azide were made as early as 1891 and in 1904 it was shown that sodium azide lowered blood pressure, stimulated respiration and increased heart rate. A thorough study of the pharmacological actions of sodium azide was made by Graham (11) in 1949. Since 1934, when Keilein (12) found that sodium azide interferred with cellular metabolism most of the work done with sodium azide has been confined to experiments on cellular respiration. In 1953 the utility of sodium azide as a drug for treating hypertension became realized when investigators studying the effects of this drug as an enzyme inhibitor in cancer research found that patients with coexisting cancer and hypertension showed a marked lowering of the blood pressure.

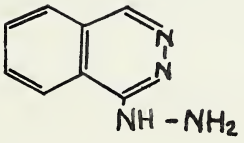
Sodium azide (NaN_3) is the sodium salt of hydrazoic acid (HN_3). It is a colorless crystalline compound which melts without decomposition and is subject to detonation at high temperatures. It is stable, neutral and soluble in water.

The pharmacological actions of sodium azide are quite complex. Its effects differ according to the species and state of the animal. The initial blood pressure, the sensitivity of its nervous system and reflex mechanism, the anaesthetic administered, and the dose and rate of administration all modify the drug's action. In general the action of sodium azide is comparable to that of sodium nitrite but the azide is far more powerful. Centrally the medulla is stimulated resulting in respiratory stimulation, an initial vagal inhibition and a subsequent sympathetic stimulation of the heart. As a result of direct stimulation the force of contraction of the isolated heart is increased. The lowering of the blood pressure is a result of direct action of the drug on the peripheral blood vessels causing vasodilation. The bronchi and coronaries are also dilated.

Sodium azide increases the tone and activity of the gut and bladder but does not alter the activity of the uterus. Large doses of sodium azide stimulate the central nervous system causing a characteristic convulsive seizure followed by depression which leads to death. Long term administration of sodium azide showed no toxic effects (13) (78).

3. Hydralazine

Hydralazine, also known as 1-hydrazinophthalazine is obtainable commercially as Apresoline (Ciba). It is usually used in the form of the hydrochloride and has the following structure:



The salt is a pale yellow-white crystalline compound with a melting point of 270-280° C. Its solubility in water is 1 in 20.

The only important pharmacological effects of hydralazine are upon the cardiovascular system (9)(16). It produces a decrease in blood pressure due to widespread vasodilation. It also produces an increased heart rate, stroke volume and cardiac output (14)(15). The cardiac effects are independent of changes in blood pressure and usually occur prior to or without vascular changes. There is an increased splanchnic and renal blood flow. This unique action of hydralazine of increasing the renal blood flow whether or not there is a fall in blood pressure prompted the extensive studies of this drug as an agent in hypertension. Although cerebrovascular resistance is decreased, cerebral blood flow is unchanged. Coronary vasodilation occurs. Both the vascular and cardiac effects of hydralazine appear to result from central vasomotor actions of the drug. There is some evidence that the drug antagonizes certain pressor substances (17)(18).

Hydralazine has only limited usefulness as an antihypertensive agent by itself but it has found some use in combination with other antihypertensive agents, chiefly the ganglionic blocking agents.

Side effects limit the drug's usefulness. Headache, nausea, postural hypotension and nasal congestion occur frequently. Chronic administration of hydralazine may produce an acute rheumatoid state which if continued, may result in a syndrome indistinguishable from disseminated lupus erythematosus (19)(20). Tolerance to hydralazine has also been reported.

III. LITERATURE SURVEY

1. Production of Hypertensive Animals

Over a hundred years ago Richard Bright made the observation that hypertrophy of the heart and hypertension were associated with chronic renal disease. Since it was generally conceded that in order to study a disease thoroughly one must first reproduce the disease in animals many attempts were made to produce a sustained hypertension by manipulation of the kidneys. Goldblatt (21) succeeded in producing sustained hypertension in dogs by applying a silver clamp to the renal artery. Modifications of this method have been used to produce hypertension in smaller animals (22). Other methods of producing experimental renal hypertension include wrapping the kidney in silk (23) or in molded rubber latex capsules (24). A method suggested by Grollman (33) for use in rats consists of passing a cotton thread around the poles of the kidney and thus producing renal ischemia. This method has been used for screening drugs for possible antihypertensive activity (25)(26).

Other methods which do not involve manipulation of the kidney can be used to produce a persistent hypertension in animals. Best and Hartroft (27) produced hypertension by subjecting weanling rats to a short period of choline deficiency. Their findings were substantiated with moderate success by some workers (30)(31). Other researchers could not obtain substantial persistent high blood pressure using this method (28)(29)(32).

Selye's (34) original description of the experimental syndrome of steroid hypertension opened a new approach to the study of experimental hypertension. Selye stated that the administeration of desoxycorticosterone acetate (DCA) to unilaterally nephrectomized rats receiving salt in their diet

produced hypertension and vascular changes similar to those observed in malignant hypertension in man. This method afforded investigators a technique by which they could more easily study many aspects of the hypertensive disease. It is necessary that sodium chloride be given concurrently with DCA. The salt is usually administered as a 1% solution as the drinking water and the DCA is injected subcutaneously or implanted in the form of pellets (35)(36)(37)(38).

The hypertension which ensues reaches a maximum after six weeks and in this stage is said to resemble essential hypertension (41). If the treatment is allowed to continue for a period up to three months a permanent hypertension results which is characterized pathologically by cardiac and renal hypertrophy and diffuse arteriosclerosis (39)(40)(41). According to Green (41)(45) the DCA cardiovascular disease in both its morphological and functional characteristics resembles the human disease more than any other type of experimental hypertension. DCA-salt induced hypertension has been used in testing the actions of various antihypertensive drugs (42 to 49).

2. Tissue Respiration of Hypertensive Animals

It has been postulated that experimental hypertension in its early stages is due to the release of pressor agents from the kidney. Although considerable attention has been focused on renin and its ability to form hypertension from plasma globulin, the existence of amino acid decarboxylases in the kidney and their ability to form pressor amines from aromatic amino acids has also been recognized. It is possible that such pressor amines contribute to the elevation of blood pressure in experimental hypertension.

There exist two mechanisms for the breakdown of amino acid by kidney tissue. In the presence of oxygen or a suitable hydrogen acceptor, L-amino acid oxidase dehydrogenates amino acid into imino acids which decompose into keto acids and ammonia. The products of amino acid oxidase activity have no effect on the blood pressure. In the alternative pathway for the breakdown of amino acids, amino acid decarboxylases convert amino acids to corresponding amines, some of which are powerful pressor substances (tyramine, hydroxytyramine, and tryptamine). The decarboxylation of amino acids is followed by oxidative deamination to aldehydes due to the action of amine oxidase.

Since in vitro amine oxidase requires oxygen in order to inactivate pressor amines it was thought that the restriction of renal circulation in experimental hypertension might make it possible for some pressor amines to escape unoxidized from the kidney and cause general peripheral vasoconstriction. It is not known whether constriction of the renal artery to the extent necessary for production of hypertension creates enough deficiency of oxygen to prevent or decrease the action of amine oxidase.

Studies on the oxygen consumption of tissues from hypertensive animals have given controversial results. Levy, Light and Blalock (52) found that the renal blood flow and oxygen consumption of the kidney were reduced following constriction of the renal arteries for 6-73 days. They also showed (53) that alterations in renal oxygen consumption usually paralleled changes in renal blood flow. Mason, Evers and Blalock (54) and Mason, Robinson and Blalock (55) showed that the renal arteriovenous oxygen difference in dogs with partial constriction of renal artery with and without hypertension was similar to normal dogs.

They found that in isolated cortical tissue from dogs and rabbits following renal arterial constriction and subtotal nephrectomy there was no diminution in the capacity of tissues from the hypertensive animal to use oxygen. Gerbi, Rubenstein and Goldblatt (51) compared the oxygen consumption of normal rabbit kidney and kidney rendered ischemic by application of a clamp to the renal artery three hours to six days prior to respiration experiments. Using tissue slices and conventional Warburg apparatus they found that the ischemic kidney showed a definite diminution of oxygen consumption compared with the normal kidney.

Raska (56) studied the oxygen consumption of slices of kidney from dogs made hypertensive with a renal clamp. He found that the respiration of the ischemic kidney was much lower than the normal kidney. He stated that a direct relationship existed between the degree in oxidizing power of the tissues. Later, Raska (57) investigated the intermediary metabolism of kidney in experimental hypertension produced by silk perinephritis or clamping of the renal artery. Using tissue slices, homogenates and extracts he found that the concentration of cytochrome c, and the activity of succinic dehydrogenase and cytochrome oxidase were markedly reduced when the preparations were obtained from hypertensive dogs.

Using mice, Cruz-Coke and Niemeyer (58) found that the oxygen uptake was the same in normal and ischemic kidney but that in the presence of succinate or lactate the uptake was less in the ischemic kidney.

Ruskin, Hall, Ruskin and Hall (60) studied the succinic dehydrogenase activity in the kidney, heart and liver of rats made hypertensive by constricting the left renal artery. They found that the ipsolateral kidney showed losses in succinic dehydrogenase activity. They also found

a moderate decrease of succinic dehydrogenase in the enlarged heart of the hypertensive animal. The contralateral kidney and liver showed no alteration of succinic dehydrogenase activity.

Lamperi and Cambiaggi (61) found a decrease in succinic and cytachrome oxidase activity in hypertensive kidney tissue of rats which had been subjected to kidney ligature.

Olsen, (59) using broken cell preparations of tissues from normal and hypertensive rats, found that there was no difference in the oxygen consumption of the liver but that the constricted kidney of the hypertensive rat had a lower oxygen consumption than its contralateral kidney or the normotensive controls. He also showed that the oxidation of amino acids and amines was depressed in the constricted kidney. These experiments were done using rats made hypertensive by the Goldblatt technique.

3. The Effects on Tissue Respiration of the Drugs Used

There has been very little reported in the literature regarding the effects on tissue respiration of the drugs used in this investigation.

(i) Desoxycorticosterone Acetate (DCA)

When fairly large concentrations of desoxycorticosterone or DCA are tipped into Warburg flasks containing slices or homogenates of rat kidney or brain a decreased oxygen consumption is noted (62)(63). The oxidative activity of kidney slices from rats treated with DCA with or without sodium chloride in the diet was studied by Knowlton et al (38). These workers found no difference between the oxygen uptake of tissues from DCA treated and normal rats. However they based their QO_2 on dry weight and the tissues used were frozen for an unstated period of time before their oxygen uptake was recorded.

(ii) Reserpine, Hydralazine and Sodium Azide

It has been stated by Lewis (3) that"it is possible that the mode of action of reserpine is that it exerts a depressant effect on some biochemical system which is concerned with respiration or some other aspect of the cell's economy." Although much work has been done regarding the activity of serotonin and reserpine, little work has been carried out in the field of tissue respiration with reserpine. In vitro studies show that reserpine depresses the oxygen consumption of slices of cerebral cortex of rat, but does this only in very high concentrations (69). In hypertensive patients, reserpine has no significant influence on glucose or oxygen consumption of the brain (70)(71).

Cerebral oxygen utilization (9) and cardiac metabolic rate for oxygen (72) remain unchanged when hydralazine is administered to patients.

Sodium azide is known to be a metabolic inhibitor (73). Keilin has shown that it interferes with enzymatic action in a manner similar to cyanide. It is known to interfere with cytochrome oxidase, catalase, peroxidase and possibly transphosphorylation. Because of its observed effects as an antihypertensive agent and metabolic inhibitor it was thought that this drug would provide an interesting tool for biochemical investigation of the pathogenesis of hypertension.

4. The Effects of the Antihypertensive Drugs in Experimental Hypertension

Although many workers have delt with the short term effects of the various antihypertensive drugs in experimental hypertension,(26)(25)(75)(76) little work has been done concerning long term evaluation of the possible protective effect of drugs commonly used in the treatment of clinical hypertension.

Black, Zweifach and Speer (78) found that sodium azide produced a sustained lowering of the blood pressure toward normal levels in hypertensive patients. Some patients were treated up to two years. They also found that sodium azide when administered intravenously to hypertensive rats lowered the blood pressure. Grizzle (13) showed that sodium azide did not effect the blood pressure of normal or Grollman hypertensive rats when given a 0.025% solution as drinking water for 40 days. In this work only 5 rats constituted a series and the worker failed to obtain significant elevations of blood pressure.

Sturtevant (44) reported that hexamethonium, hydralazine, and Veriloid reduced blood pressure transiently after single injections in DCA hypertensive rats. He also found that oral feeding of crude Rauwolfia would reduce pressure during a short period of observation. Gaunt et al (49)(42) showed that prolonged administration of reserpine and hydralazine lowered blood pressure, reduced the extent of pathological changes and lengthened survival time of DCA hypertensive rats. Gross, Noelpp, Sulser, Doebelin and Kundig (46) showed that reserpine and hydralazine reduced blood pressure in rats which had been made hypertensive by several methods.

IV. STATEMENT OF THE PROBLEM

Previous reports regarding the respiration of tissues from hypertensive animals have presented conflicting results. Many of these discrepancies may have been due to the fact that different workers suspend tissues in different media, each worker considering certain metabolites essential. It is recognized that this does not represent an *in vivo* situation. Huston and Martin (79) have proposed a new technique whereby the tissues to be studied are excised, spread on fibre glass mats and then placed in an atmosphere of oxygen superimposed above fluid medium in a special type of Warburg flask. Since then, modifications of this technique have been used successfully (77)(80)(81). It is felt that this technique would more closely approach the *in vivo* situation.

In previous work the animals used were made hypertensive by placing a clamp on the renal arteries or by constricting the kidney itself. By producing hypertensive rats by several methods and measuring the oxygen uptake of heart and kidney tissue using the new procedure and comparing it with the effects in the conventional Warburg flasks it was hoped to clarify the existing situation. In addition it was the purpose of this work to test the effects of certain antihypertensive drugs on the blood pressure and tissue respiration of normal and hypertensive rats. The drugs chosen were reserpine, hydralazine and sodium azide.

V. EXPERIMENTAL

1. Production of Hypertensive Animals

In most of the previous work on tissue respiration of hypertensive animals, the high blood pressure was produced by applying a clamp to the renal arteries. It was decided to develop hypertensive animals by several other methods. Three methods of producing hypertension in the rat were chosen:- choline-free diet, Grollman method and DCA-salt method.

(a) Choline-Free Diet

The choline-free diet was chosen as it was thought that a true hypertension could be produced without manipulation of the kidney or an operation of any kind. The method used was that of Handler and Bernheim (31). Male weanling rats weighing 40 to 60 grams were fed a basic choline-free diet for six days. After this period the rats were maintained on a regular diet. The formula of the choline-free diet was as follows:-

casein	15.0
sucrose	48.6
cotton seed oil	15.0
lard	10.0
cod liver oil	5.0
cysteine	0.4
cholesterol	1.0

(b) Grollman Method

Since this method was first reported (33) many workers have used it with success. As it is a proven method and is relatively simple it was chosen as the second method of producing hypertensive rats.

The method consists of passing a cotton thread around the poles of one kidney in a figure of eight fashion, pulling it taut so as to ensure some compression of the organ, and tying it securely. The same procedure is followed with the other kidney after a period of 10 days. Such a method produces a progressive hypertension.

Male rats weighing 100-150 grams were first anesthetized with 25 mg./Kg. of sodium pentobarbital. The hair on the left lumbar region was shaved and the skin painted with Tincture of Metaphen. An incision was made starting just below the ribs and extending distally about one inch, parallel to and one half inch from the spinal column. The underlying dorsal muscle was separated by blunt dissection to expose the kidney. This organ was then pulled through the incision, the adrenal gland and perirenal fat carefully separated and replaced in the body cavity. The kidney itself was decapsulated. A cotton thread was placed around the two poles of the kidney in figure eight fashion and tied securely. The kidney was then gently pushed back into place, and the incision closed with wound clips and Tincture Metaphen again applied.

(c) DCA Method

The method of producing hypertensive animals by the hormonal or DCA method was chosen because it is relatively predictable in its course and reproducible in its occurance (35). Since this type of experimental hypertension most closely resembles essential hypertension in man (41) it was also decided to use this method in testing the effects of the antihypertensive drugs.

Male rats weighing 70 to 150 grams were anesthetized with sodium pentobarbital (25 mg./Kg.) The left kidney was exposed in a manner similar

to that in the Grollman method and was then removed. One pellet containing 75 mg. of DCA was implanted in the scapular region by means of a pellet injector. The incision was closed with wound clips and Tincture of Metaphen applied. The rat was given 1% sodium chloride solution as drinking water thereafter.

2. Measurement of Blood Pressure

The photoelectric tensometer (86) is designed to determine the blood pressure of small animals without anesthetizing or heating the animal. The principle of its use is based upon measurement of the volume change in the foot by the use of a photoelectric cell, before and after application of pressure by means of a miniature sphygmomanometer cuff applied to the ankle.

The unanesthetized rat was placed in a special holder which allowed the hind legs to hang free. The holder was clamped to a mounting rod so that the animal's body formed a 45 to 60 degree angle with the surface of the photocell box. A small rubber cuff was wrapped around the right hind ankle and held securely by a ribbon and a clamp. The cuff was attached to a sphygmomanometer. The foot was then placed in a holder which was so constructed that it was directly above the photocell. The light from a special flashlight was brought directly over the foot and above the photocell. The cuff was then inflated to 250 mm. Hg. which restricted the arterial flow of blood into the foot. At this point the circuit resistance control was adjusted so that the milliammeter which records the light intensity on the photocell recorded approximately 0.7 milliamperes. The pressure was slowly lowered in the cuff (approximately 280 mm. Hg. to 0 mm. Hg. in 30 seconds). When the arterial blood flow into the foot was again resumed the volume of blood in the foot increased

and thus the intensity of light reaching the photocell was reduced. At the exact instant that the milliammeter needle dropped the sphygmomanometer was read. This reading was taken as the systolic blood pressure of the animal.

3. Determination of Tissue Respiration

(a) Apparatus and Solutions Used

In order to measure the respiration of tissues in contact with oxygen, it is necessary to have the samples suspended in such a manner as to assure maximal contact of the tissue with the gas. The slices of tissue were spread out evenly on fibre glass mats and placed in wide mouthed flasks designed by Huston and Martin (79).

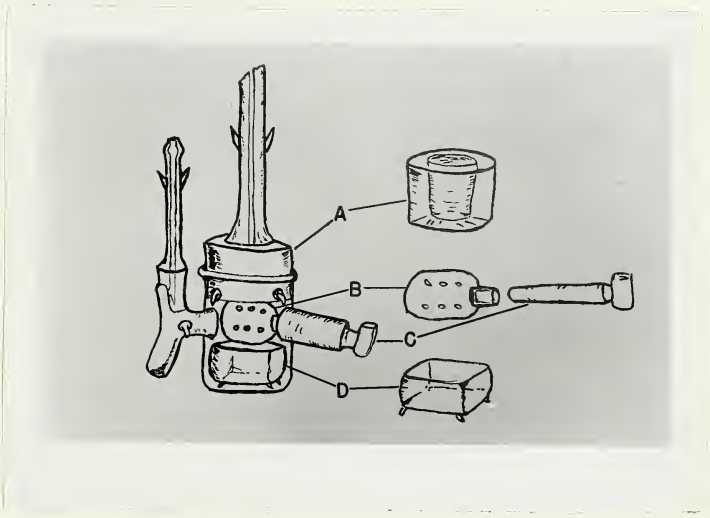


FIGURE I - HUSTON-MARTIN FLASK

Figure 1 is an illustration of the flask. The vessel, of about 19 ml. capacity, is attached to a standard Warburg manometer by a glass

adapter (A). A removable tray (D) rests on the bottom of the vessel. The mat bearing the tissue is placed on a rotatable paddle (B,C) above the tray.

Since it was desirable to compare the respiratory rates of tissues suspended in oxygen with those found by more standard procedures, samples of each organ were also investigated in liquid media. Regular Warburg flasks were used for this purpose. Two liquid media were used; Krebs Ringer Phosphate (82), a simple saline solution, and Krebs Medium III (83) a fortified solution. The formulae for these solutions are as follows:-

(i) Krebs Ringer Phosphate (KRP)

0.9% (0.154M) NaCl 100

1.5% (0.154M)KCl 4

1.22% (0.11M) $CaCl_2$ 3

2.11% (0.154M) KH_2PO_4 1

3.82% (0.154M) $MgSO_4 . 7H_2O$ ... 1

0.1M Phosphate Buffer pH 7.4 .. 12

{ 17.8 g. $Na_2PO_4 \cdot H_2O$; 20 ml. 1N HCl } diluted to 1 Litre

(ii) Krebs Medium III (KMIII)

0.9% (0.154M) NaCl 95

1.15% (0.154M) KCl 4

1.22% (0.11M) $CaCl_2$ 3

2.11% (0.154M) KH_2PO_4 1

3.82% (0.154M) $MgSO_4 \cdot 7H_2O$ 1

1.3% $NaHCO_3$ 3

Na Phosphate Buffer 3

{ 0.1M Na_2HPO_4 (1.78% $Na_2HPO_4 \cdot 2H_2O$) ... 100 ; 0.1M NaH_2PO_4 (1.38% $NaH_2PO_4 \cdot H_2O$) .... 25 }

0.16M Na Pyruvate 4

0.1M Na Fumarate 7

0.16M Na-L-Glutamate 4

0.3M Glucose 5

Since the metabolites present in KMIII cannot be stored in solution longer than a week, they were freshly prepared each week. Concentrated stock solutions of salts were prepared and kept under refrigeration, being diluted before use and added to metabolites.

(b) Method of Handling Animals and Tissues

Male albino rats of Wistar strain were used throughout the experiment. The Grollman type hypertensive animals were used 5 to 11 weeks after the initial operation. The DCA hypertensive animals were used 40 to 50 days following implantation. When antihypertensive drugs were used, treatment was begun at the time of DCA implantation and continued until the animal was killed. Only DCA hypertensive and normal rats were treated with the drugs.

Reserpine was injected subcutaneously once daily, six times a week at a dosage of 0.1 mg./Kg. Apresoline was given in the drinking solution and adjusted to achieve an approximate intake of 0.1 mg./Kg. daily. Sodium azide was administered in 0.025% solution as drinking water. Each rat received approximately 3.5 mg./100 g. over a 24 hour period.

After first determining the blood pressure the rat was killed by a blow on the head. The animal was placed in a cold (2-5° C), moist chamber where the tissues to be investigated were quickly excised. Heart was sliced through a template and kidney by a Martin slicer (84).

As the tissues were sliced they were placed upon tared pieces of waxed paper or tared fibre glass mats. These were kept in moist petri dishes in the refrigerated cabinet until all sections were ready for weighing. The weighing was done a Gram-atic balance and the tissues immediately removed to the Warburg flasks. Two samples of each tissue were placed in KRP, two in KMIII and two in the Huston Martin flasks (HM flasks). The tissues spread out on the fibre glass mats were placed on the paddles in the HM flasks and those on waxed paper removed to the fluid media. After attachment to the manometers, the flasks were placed in the constant temperature water bath at 37.9° C where oxygenation was carried out for two minutes. Fifteen minutes was allowed for thermal equilibration. The operation and weighings required from 25 to 30 minutes. Measurement of respiration was by the direct method of Warburg at a shaking rate of 120 cycles per minute.

For CO_2 absorption 0.2 ml. of 10% potassium hydroxide solution was placed in the centre well of the standard flasks and was absorbed into filter paper discs placed on the bottom of the HM flasks. Each flask received 1.5 ml. of solution, this being contained in the removable tray (Fig. 1-D) of the HM flasks. Readings were taken every ten minutes for the first hour and every twenty minutes for the second hour.

(c) Calculation of Results

Warburg manometers record changes in pressure of gas in the flask. The flask constant, coupled with the weight of the tissue is used to determine a standardized QO_2. The QO_2 in this investigation expresses ml. of oxygen consumed per gram of tissue (wet weight) per hour. Since the rate of respiration in artificial medium declines with time, the QO_2 value for zero time must be obtained by straight line extrapolation of

the rates during the experimental period. The rate of respiration has been depressed by the cold during the operation procedure and returns to a maximum at the conclusion of thermal equilibrium. This rate of respiration is therefore the closest approximation to that *in situ*. The QO_2 value at sixty minutes after thermal equilibration is not comparable to that in the intact animal but merely gives an indication of the rate of decline of the respiration of the tissues under artificial conditions. For this reason the sixty minute QO_2 values were recorded.

Mean QO_2 values of each tissue investigated were determined. Any difference from normal values noted was tested for significance by the Student's "t" test (85). The probability of 0.01 was selected as the point of significance.

VI. RESULTS

1. Development of Hypertensive Rats

The mean blood pressure of normal animals was found to be 117 mm. Hg (Table IV).

(a) Choline-Free Diet

Seventeen male rats weighing approximately 50 grams were fed a choline-free diet for six days and then left on a normal stock diet for the remaining period of the experiment. Fourteen rats survived the diet and thirteen were living after seven months. The average blood pressure of this group was 117.2 mm. Hg with the highest blood pressure being 134 mm. Hg.

Nine male rats weighing approximately 50 grams were fed a choline-free diet for six days and then left on a stock diet thereafter. A one percent sodium chloride solution was substituted for the drinking water for three months, after which time a two percent saline solution was given the rats. All rats survived the diet. Seven rats were living after four months, at which time the average blood pressure was 129 mm. Hg with one rat having a blood pressure of 170 mm. Hg. After seven months only three rats were surviving. These animals had an average blood pressure of 113 mm. Hg.

(b) Grollman

Rats made hypertensive by the Grollman method were used for tissue respiration studies five to eleven weeks after the second operation. At this time a substantial progressive hypertension had resulted. The mean blood pressure of nine rats studied was 183 mm. Hg with a range of 150-220 mm. Hg (Table II).

(c) DCA

Rats made hypertensive by the DCA method were sacrificed for respiration studies forty to fifty days after implantation. At the time of sacrifice eight rats studied had a mean blood pressure of 219.5 mm. Hg with a range of 182 - 280 mm. Hg (Table III). Only eight of the thirteen rats or 61.6% of those initially implanted survived the waiting period.

2. Effects of Antihypertensive Agents on the Blood Pressure of Normal and DCA Hypertensive Rats

(a) Reserpine

Normal rats treated with reserpine for a period of forty to fifty days had a mean blood pressure of 113 mm. Hg and a range of 74 - 140 mm. Hg (Table VII). All the normal rats so treated survived this waiting period. DCA implanted rats treated with reserpine had a blood pressure of 171 mm. Hg and a range of 112 - 234 mm. Hg (Table VIII) at the time of killing. Of eleven rats implanted, 9 rats (81.8%) survived the waiting period. All rats treated with reserpine gained weight and appeared to be in good condition at the time of sacrifice. It was noted that the DCA implanted rats treated with reserpine drank less saline than the untreated DCA implanted rats.

(b) Sodium Azide

The mean blood pressure of DCA implanted rats treated with sodium azide for forty to fifty days was 172 mm.Hg with a range of 102 - 240 mm. Hg (Table X). Eleven of fourteen rats (78.6%) implanted survived the waiting period. All normal rats treated with sodium azide for the same period survived and had an average blood pressure of 118 mm. Hg with a range of 108 - 128 mm. Hg at the time of sacrifice (Table IX).

The normal animals gained weight and were fairly active during the treatment while the DCA implanted animals gained little weight and appeared very ill at the time of killing. DCA implanted rats drank less of the solution containing 0.025% sodium azide and 1% sodium chloride than the DCA implanted rats drinking 1% sodium chloride solution.

(c) Hydralazine

The mean blood pressure of the DCA implanted rats treated for a period of forty to fifty days with hydralazine was 154 mm. Hg with a range of 118 - 178 mm. Hg (Table XII). Normal rats treated with hydralazine over the same period had a mean blood pressure of 114 mm. Hg with a range of 102 - 122 mm. Hg (Table XI). All rats, both normal and DCA implanted survived the waiting period. During this time of treatment the rats in both series were active and gained weight. No difference was noted in the consumption of saline by hydralazine treated DCA hypertensive rats and untreated DCA hypertensive rats.

The effects of the antihypertensive agents on the blood pressure of the experimental animals is shown in Figure II. The blood pressures of the individual animals are shown in the respective tables along with the QO_2 values. It should be noted that rats were considered hypertensive when they had a blood pressure greater than 150 mm. Hg.

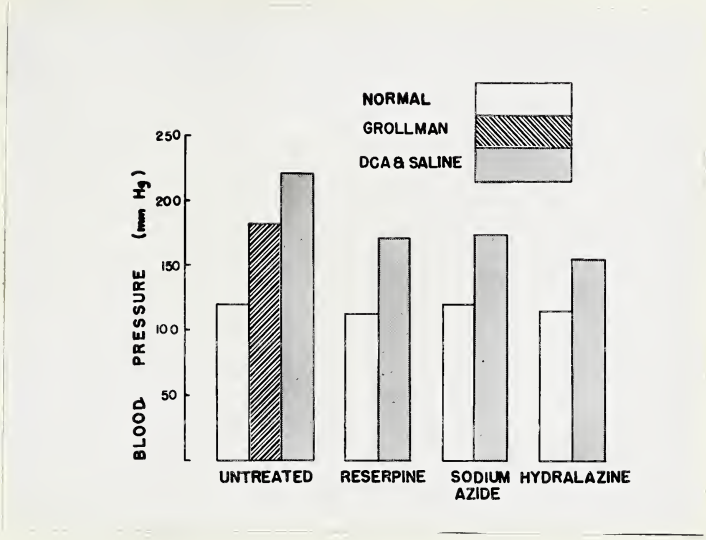


FIGURE II

The Effects of Antihypertensive Agents on
Blood Pressure of Normal and Hypertensive Rats

3. Tissue Respiration of Untreated Normal and Hypertensive Rats

(a) Kidney

(i) Normal Kidney

Table I shows the normal values obtained for rat kidney in KRP, KMIII and HM flasks at zero time and sixty minutes thereafter. The mean QO_2 values at zero time were 3.53 in KRP, 4.32 in KMIII and 4.70 in HM flasks. The low value in KRP, higher in KMIII and the even higher value obtained in HM flasks is of interest, and substantiates the findings of Huston and Martin (79). Although the QO_2 value in KMIII is higher than they report the value in HM flasks compares very well with their QO_2 value (4.68) and with that (4.71) reported by Brody (81).

(ii) Grollman Hypertensive Kidney

The kidney removed from the Grollman hypertensive animal was usually enlarged and greenish-yellow in color. The tissue was very fibrous and often contained pockets of pus. At the time of sacrifice several rats were passing blood in the urine. It was difficult to obtain good slices from these kidneys and the time to cut them (30 to 50 minutes) was usually longer than was required to cut normal kidney tissue. The mean QO_2 values at zero time were 2.82 in KRP, 2.98 in KMIII and 3.24 in HM flasks. These values are all significantly lower than those obtained from normal kidney. The results are presented in Table II.

(iii) DCA Hypertensive Kidney

The kidney removed from the DCA hypertensive animal was enlarged and of a motley brown-yellow color instead of the normal maroon color. The tissue was not fibrous but rather firm and moist. Slices were easy to cut. The mean QO_2 values of the kidney from the DCA hypertensive rat

were all decreased significantly from the normal values. The mean QO_2 values at zero time were 2.57 in KRP, 2.89 in KMIII and 2.95 in HM flasks. The individual QO_2 values at zero and sixty minutes are recorded in Table III.

The kidney from either type of hypertensive rat had a lower QO_2 value both at zero time and sixty minutes than those of normal rats. This was true both in the conventional Warburg and the HM flasks.

(b) Heart

(i) Normal Heart

Samples of heart were taken from the same animals from which the kidney slices were obtained. Table IV shows the QO_2 values at zero time and sixty minutes thereafter for normal rat heart. Like kidney, the QO_2 values in HM flasks were higher than the values from KMIII and KRP. Thus KRP has the lowest QO_2 values for both heart and kidney and HM flasks the highest at both zero time and at sixty minutes afterward. The QO_2 values for heart at zero time in KRP was 1.00; in KMIII it was 1.79 and in HM flasks was 2.73. These values are all slightly lower than those reported by Huston and Martin (79). It would seem that KRP is a poor medium for the study of rat heart respiration as the values obtained were very low and inconsistent when compared with the HM flasks or KMIII.

(ii) Hypertensive Heart

The heart from both the DCA and Grollman hypertensive rat was larger and more fibrous than the normal heart. There was a significant decrease at zero time of the QO_2 values in KRP and HM flasks. The QO_2 values for the heart from Grollman hypertensive rats are shown in Table V while those values from DCA hypertensive rats are in Table VI.

Figure III shows the graphical comparison of the respiration of the heart and kidney from DCA and Grollman hypertensive rats with that of normal rats.

TABLE I

RESPIRATION OF NORMOTENSIVE RAT KIDNEY SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	114	3.50	4.80	4.60	3.38	3.67	4.26
		3.80	5.10	4.69	3.65	2.64	4.24
	108	3.81	4.64	4.74	3.39	2.92	4.16
		3.79	4.92	4.28	3.30	3.54	4.00
	126	3.30	4.95	4.28	2.79	3.85	3.88
		3.50	4.48	4.59	3.02	3.42	4.45
	114	3.62	4.28	4.40	2.88	3.48	4.37
		3.56	4.77	4.90	2.94	3.78	4.75
	116	3.58	4.66	5.45	2.76	3.59	4.77
		3.87	4.64	5.36	3.14	3.72	4.48
	106	3.80	4.52	4.59	2.64	3.90	4.13
		3.54	4.50	5.62	2.67	3.83	4.81
	126	3.36	3.63	4.69	2.68	3.44	4.63
		3.24	3.76	4.87	2.67	3.30	4.76
	112	3.82	3.88	4.00	3.46	3.32	4.37
		2.75	3.80	-	2.66	3.28	-
	120	3.23	3.48	4.64	3.13	2.83	4.55
		3.50	3.72	-	3.35	3.22	-
	120	3.33	3.71	4.88	3.11	3.12	4.76
		3.14	3.92	4.68	3.03	3.22	4.33
	120	3.84	4.50	4.59	3.25	3.02	3.86
		-	3.78	3.62	-	2.83	3.41
	116	3.86	4.72	4.66	3.70	3.35	4.50
		-	4.63	4.81	-	3.10	4.58
mean	116.5	3.53	4.32	4.70	3.08	3.35	4.37
s.d.m.		0.283	0.485	0.411	0.320	0.344	0.348

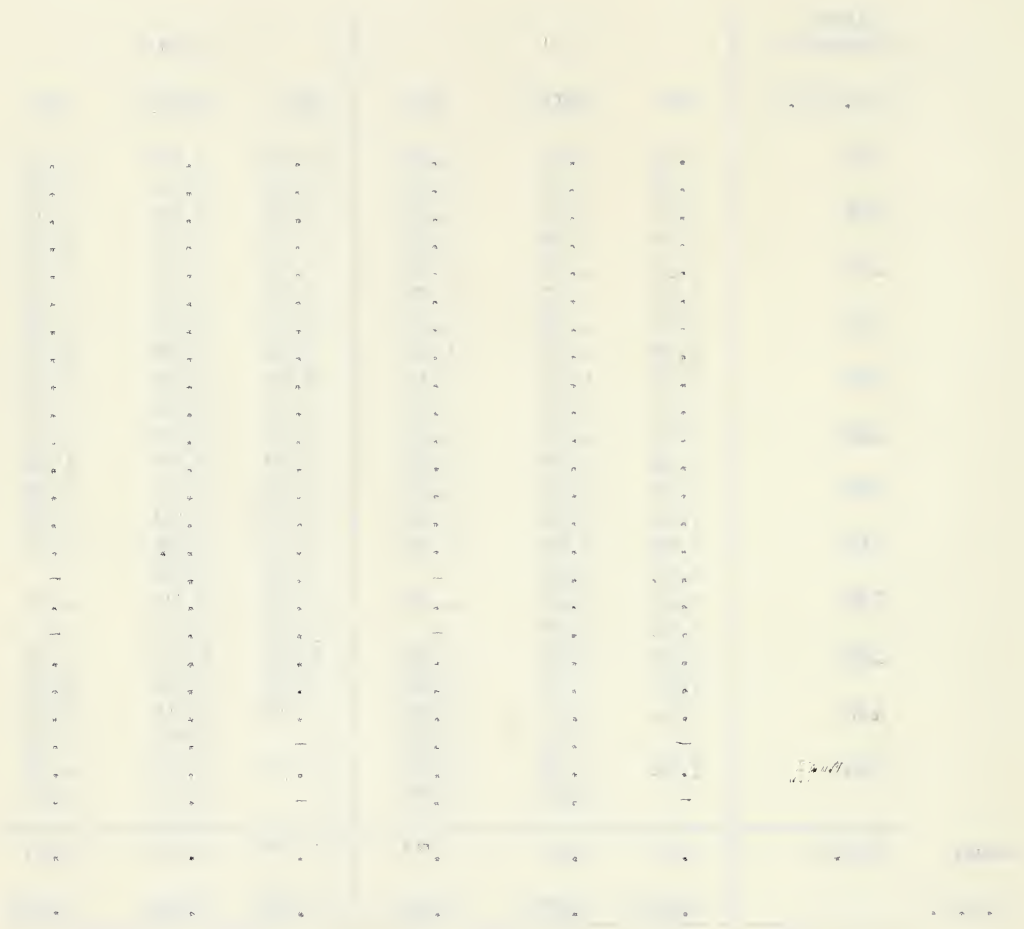


TABLE II

RESPIRATION OF GROLLMAN HYPERTENSIVE RAT KIDNEY SLICES

Figures represent Q_{O_2} values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	200	2.62	3.06	2.56	1.98	2.28	2.52
		-	3.20	-	-	2.26	-
	180	3.68	3.68	3.42	2.16	3.02	3.02
		4.20	2.59	3.15	2.72	2.11	3.05
	150	2.04	2.40	-	1.64	1.97	-
		2.71	2.43	-	2.30	2.07	-
	180	3.24	3.91	4.40	2.86	2.47	3.94
		3.40	3.30	3.12	2.98	2.20	2.64
	220	2.38	2.40	-	2.34	1.66	-
		2.23	2.80	-	2.08	1.72	-
	218	2.09	3.30	2.75	2.06	1.91	2.69
		2.27	2.94	-	2.17	2.06	-
	180	2.29	1.80	3.49	1.83	1.40	3.28
		2.29	2.26	2.20	1.83	1.63	2.13
	162	3.22	2.16	2.99	2.85	1.23	2.43
		-	3.52	2.37	-	1.72	2.10
	160	2.93	3.86	4.02	2.62	3.23	3.62
		3.48	3.87	4.39	3.29	3.02	4.14
mean	183	2.82	2.97	3.24	2.36	2.11	2.96
s.d.m.		0.631	0.633	0.707	0.462	0.537	0.644

TABLE III

RESPIRATION OF DCA HYPERTENSIVE RAT KIDNEY SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	220	1.55	2.39	2.38	1.40	2.33	2.21
		2.40	2.21	2.72	2.32	2.10	2.61
	190	2.14	2.56	2.88	2.00	2.31	2.55
		2.45	2.58	2.85	2.37	2.08	2.69
	190	3.20	3.30	3.24	2.79	1.90	3.13
		3.33	3.69	3.02	2.83	2.77	3.02
	234	2.53	1.95	3.24	2.41	1.88	2.44
		2.29	2.44	2.71	2.20	2.25	2.58
	182	2.34	2.33	3.02	2.25	2.10	2.81
		2.71	2.89	2.94	2.51	2.17	2.67
	280	2.67	3.54	3.16	2.20	2.20	2.59
		2.86	3.31	2.59	2.48	1.77	2.35
	222	2.46	3.43	2.84	2.34	1.86	2.77
		2.97	2.88	2.81	2.63	2.07	2.71
	238	2.50	3.55	3.37	2.32	2.37	2.92
		2.72	3.14	3.37	2.53	2.04	3.25
mean	219.5	2.57	2.89	2.95	2.35	2.14	2.71
s.d.m.		0.410	0.532	0.272	0.322	0.236	0.267

TABLE IV

RESPIRATION OF NORMOTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	116	-	1.82	3.07	-	0.65	1.40
		-	1.82	-	-	0.65	-
	114	1.67	1.58	2.80	0.92	0.48	2.18
		-	2.32	-	-	0.78	-
	120	0.78	1.36	-	0.33	0.42	-
		-	-	-	-	-	-
	118	0.28	1.72	2.54	0.24	0.36	1.70
		0.30	1.40	3.06	0.26	0.26	0.50
	118	0.85	1.24	2.97	0.60	0.43	1.40
		0.89	1.12	2.46	0.50	0.67	1.72
	124	1.05	2.13	3.22	0.47	0.73	1.84
		1.20	2.06	2.96	0.74	0.34	1.05
	114	1.15	2.37	2.24	0.73	0.96	1.28
		0.72	1.76	2.69	0.42	0.42	1.58
	108	1.13	1.08	2.62	0.49	0.57	1.96
		-	1.50	2.87	-	0.81	1.60
	126	-	1.25	3.28	-	0.68	1.26
		-	-	2.72	-	-	2.04
	114	0.83	-	2.40	0.64	0.26	1.96
		0.70	-	-	0.26	-	-
	-	1.20	1.44	2.31	0.87	0.40	1.54
		-	2.36	3.30	-	1.05	1.76
	116	1.12	-	2.40	0.50	-	1.80
		-	-	2.36	-	-	1.24
	106	1.21	1.88	2.58	0.56	0.76	1.90
		-	2.07	2.66	-	0.91	1.98
	126	1.01	2.24	2.75	0.45	1.00	2.03
		0.96	2.36	2.77	0.36	0.70	2.20
	112	0.99	1.92	2.62	0.76	0.57	2.14
		-	1.92	3.12	-	0.57	2.29
	120	1.06	1.27	2.41	0.96	0.54	2.41
		0.77	1.27	-	0.71	0.54	-
	120	1.31	2.12	2.52	0.80	0.95	2.30
		1.34	2.33	2.45	1.02	0.95	2.13
	120	1.13	1.54	2.85	0.56	0.20	2.23
		1.00	1.31	3.26	0.60	0.20	2.64
	116	1.37	2.39	2.42	1.25	1.11	2.23
		-	2.36	2.70	-	1.38	2.17
mean	117.0	1.00	1.79	2.73	0.62	0.63	1.83
s.d.m.		0.300	0.399	0.303	0.249	0.286	0.440

TABLE V

RESPIRATION OF GROLIMAN HYPERTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	200	0.40	1.06	1.72	0.34	0.35	0.77
		-	1.26	2.15	0.59	-	1.26
	180	0.86	1.42	2.16	0.32	0.41	1.57
		0.66	1.46	2.36	0.17	0.51	1.27
	150	0.27	1.68	1.94	0.21	1.40	1.24
		0.90	1.75	2.50	0.49	0.60	1.29
	180	0.66	1.63	2.51	0.52	0.42	1.75
		0.67	1.63	2.21	0.51	0.55	1.85
	220	0.38	1.58	2.50	0.22	1.06	1.67
		0.67	1.82	2.95	0.55	1.55	1.78
	218	0.51	1.96	2.60	0.22	0.96	1.19
		0.94	1.89	2.52	0.67	0.60	1.62
	180	0.78	1.43	2.50	0.65	0.63	2.07
		-	1.39	-	-	1.22	-
	162	1.05	1.47	2.22	0.40	0.58	1.90
		0.82	1.60	1.74	0.65	0.94	1.74
	160	1.08	2.02	1.83	0.89	0.91	1.64
		0.73	1.81	-	0.60	1.11	-
mean	183	0.71	1.60	2.28	0.47	0.81	1.54
s.d.m.		0.227	0.243	0.333	0.196	0.352	0.326

TABLE VI

RESPIRATION OF DCA HYPERTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	220	0.69	2.32	1.49	0.46	1.24	1.25
		0.45	1.75	1.64	0.27	0.48	1.30
	190	0.84	1.28	2.20	0.70	1.12	2.08
		0.84	1.24	2.30	0.30	0.75	1.06
	190	0.62	1.88	2.66	0.98	0.91	2.00
		1.23	1.97	2.55	0.50	0.75	2.08
	234	0.78	2.20	2.70	0.50	0.75	1.45
		1.28	2.20	2.92	0.93	0.80	1.95
	182	1.13	1.64	-	0.90	0.80	1.56
		1.02	1.80	2.60	0.83	0.69	1.60
	280	0.37	1.66	2.60	0.27	0.58	1.50
		0.46	1.66	2.91	0.30	0.58	1.59
	222	0.86	1.28	1.79	0.74	0.31	1.08
		0.48	1.39	2.33	0.40	0.39	1.22
	238	0.71	1.60	2.32	0.33	0.52	1.40
		0.67	1.86	2.42	0.45	0.64	1.45
mean	219.5	0.78	1.73	2.36	0.55	0.71	1.53
s.d.m.		0.270	0.326	0.417	0.246	0.237	0.348

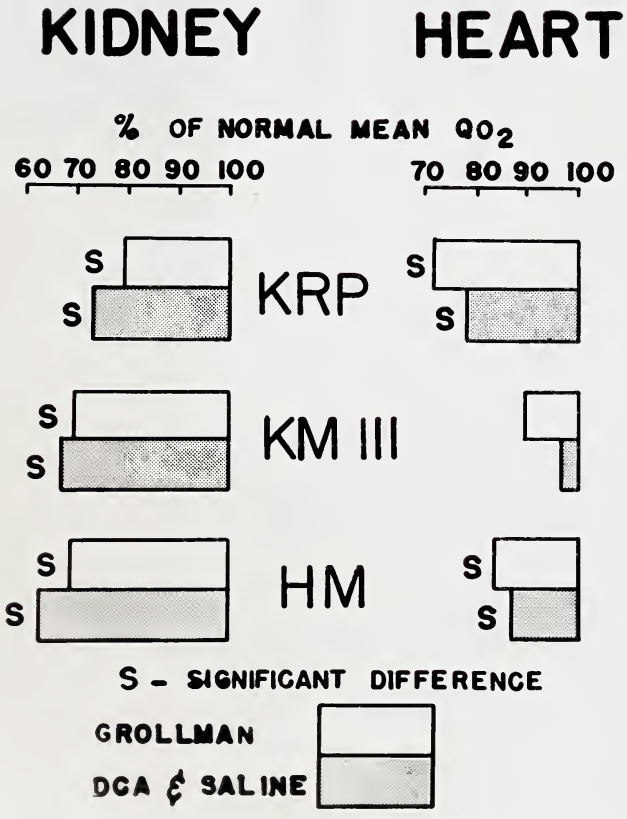


FIGURE III

Respiration of Heart and Kidney from DCA and Grollman Hypertensive Rats Expressed as a Percent of Mean Normal QO_2 Values at Zero Time

4. Tissue Respiration of Drug Treated Normal and DCA Hypertensive Rats

(a) Kidney

(i) Reserpine

*Serpasil Parenteral Solution (reserpine - Ciba) was diluted with water for injection so that 0.1 mg. of reserpine was contained in 1 ml. of injection. The animals were injected subcutaneously with 0.1 mg. /Kg. of the drug at the same time each morning. The blood pressure was taken and the animal sacrificed two hours after the last injection. At the time of sacrifice the treated rat was mildly sedated. Ptosis of the eyelid and diarrhea were observed in these animals. The kidneys removed from the hypertensive rats were enlarged but otherwise normal in appearance.

The QO_2 values for the normal and hypertensive rats treated with reserpine are shown in Tables VII and VIII. Reserpine had no significant effect on the respiration of the normal kidney. The mean QO_2 values of the hypertensive kidneys were decreased in all flasks but significantly only in KMIII. These results would seem to indicate that the reserpine protected the kidney in some way, since the respiration of the reserpine treated hypertensive kidney was not as reduced as in the untreated hypertensive kidney.

(ii) Sodium Azide

Sodium azide was administered to the rats in the drinking water as a 0.025% solution. It has been reported that at this concentration the drug was well tolerated (13). It was found that when sodium azide was added to the drinking water, less drinking solution was consumed

* Supplied by Ciba Company Limited, Montreal.

by the rats. Although the amount varied, approximately 3.5 mg./Kg. of sodium azide was consumed daily by the normal and DCA implanted rats. It should be noted that some of the very ill hypertensive animals received less than this amount during the last few days of the waiting period.

Both normal and hypertensive kidneys from sodium azide treated rats showed no gross changes from the normal kidney. Administration of sodium azide had no effect on the mean QO_2 values of kidney slices from normal animals. The mean QO_2 values of kidney tissue from hypertensive animals showed a significant increase from normal in all flasks. The QO_2 values of kidney from normal rats treated with sodium azide are presented in Table IX; the values for the DCA hypertensive kidney are shown in Table X.

(iii) Hydralazine

*Apresoline HCl (hydralazine - Ciba) was administered to the rats in the drinking water so that about 0.1 mg./Kg. was consumed daily. The presence of hydralazine did not lessen the intake of the drinking solution as did the sodium azide. Both normal and hypertensive animals gained weight and were active throughout the waiting period. It was noted that four out of the ninehypertensive rats used had enlarged brown colored kidneys while the other five had enlarged but normal appearing kidneys.

As recorded in Table XI, hydralazine had no effect upon the QO_2 of normal rat kidney. The QO_2 values of the hypertensive kidney slices were significantly decreased in all flasks (Table XII). However the mean QO_2 values were not as low as those of the untreated DCA hypertensive kidney slices.

* Supplied by Ciba Company Limited, Montreal.

TABLE VII

EFFECT OF RESERPINE ON THE RESPIRATION OF NORMOTENSIVE RAT KIDNEY SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	80	4.13	4.94	4.67	3.60	3.50	4.54
		4.06	5.14	-	3.38	2.92	-
	74	3.69	4.67	5.84	3.52	3.27	5.08
		3.84	3.56	-	3.67	3.02	-
	126	3.20	4.00	4.58	3.05	2.80	4.19
		3.43	3.39	-	2.85	3.17	-
	120	2.92	3.34	3.74	2.72	2.93	3.55
		3.74	3.67	3.81	3.42	3.08	3.26
	140	-	3.54	4.60	3.10	2.48	4.35
		3.76	3.92	4.04	2.40	3.00	3.74
	114	3.32	4.72	4.75	3.14	3.46	4.20
		3.20	4.22	4.69	3.05	2.52	4.21
	132	3.04	3.18	3.73	2.89	2.87	3.57
		3.04	3.96	-	2.89	2.84	-
	110	3.83	4.53	5.08	3.20	2.40	4.78
		3.89	-	-	3.13	-	-
	106	3.62	5.34	5.36	3.44	3.24	5.08
		4.31	5.41	5.21	4.11	3.68	4.87
	108	3.70	4.38	5.84	3.60	3.82	4.80
		4.04	4.46	5.48	3.38	3.04	4.52
	122	3.74	4.26	4.79	3.40	3.85	4.33
		4.09	3.92	5.04	3.68	3.38	4.37
	120	3.24	3.86	5.15	3.14	3.07	4.70
		3.33	4.14	4.62	3.25	3.08	4.16
	110	3.49	4.60	4.84	3.32	3.08	4.29
		3.68	3.92	4.66	3.49	3.32	4.23
	114	4.08	5.14	5.52	3.65	3.97	4.34
		3.82	4.04	4.66	3.26	3.71	3.91
	118	4.42	4.52	4.79	4.04	3.15	3.97
		3.15	4.03	4.95	2.54	3.24	4.00
mean	113	3.65	4.23	4.82	3.28	3.17	4.28
s.d.m.		0.395	0.590	0.563	0.385	0.385	0.455

TABLE VIII

EFFECTS OF RESERPINE ON RESPIRATION OF DCA HYPERTENSIVE RAT KIDNEY SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	160	3.88	4.28	-	3.14	2.38	-
		3.88	-	-	3.14	-	-
	174	3.63	4.32	5.32	3.34	3.80	4.85
		4.04	5.25	5.24	3.77	4.07	4.42
	144	3.54	4.16	4.41	3.20	2.80	3.92
		3.43	4.50	4.72	3.13	2.80	4.20
	112	2.92	2.97	4.64	2.86	2.75	4.47
		3.18	3.35	4.73	3.04	2.94	4.69
	158	3.91	3.86	4.49	3.59	2.80	4.07
		4.18	3.10	4.27	3.78	2.18	4.02
	234	3.07	3.22	3.23	2.64	2.16	2.89
		3.19	3.42	3.36	2.75	2.23	3.18
	198	2.93	3.69	3.62	2.63	2.86	2.96
		2.93	3.46	3.57	2.71	2.83	3.01
	188	3.38	3.95	4.47	3.15	2.77	3.86
		4.34	4.84	4.12	3.75	2.96	3.52
mean	171	3.53	3.81	4.30	3.16	2.82	3.86
s.d.m.		0.442	0.643	0.631	0.390	0.514	0.632

TABLE IX

EFFECTS OF SODIUM AZIDE ON THE RESPIRATION OF NORMOTENSIVE RAT KIDNEY SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	128	3.61	4.25	5.11	3.00	2.84	4.36
		4.20	4.45	4.97	3.62	3.50	4.71
	122	3.55	4.06	4.85	3.41	2.92	4.82
		3.65	4.19	4.97	3.42	1.63	4.94
	128	3.47	4.54	6.27	3.16	3.26	5.22
		3.63	4.59	5.16	3.49	3.38	4.96
	118	-	4.20	-	-	3.54	-
		-	4.54	-	-	3.92	-
	114	3.66	4.50	4.09	3.22	2.76	3.45
		3.66	3.93	4.90	3.40	3.02	3.94
	108	3.41	4.62	4.49	3.26	3.90	3.60
		3.47	4.65	-	3.29	4.00	-
	116	3.62	4.29	4.28	3.43	3.39	3.58
		3.68	4.03	4.11	3.42	2.93	3.95
	110	3.40	4.30	4.45	3.26	2.90	3.94
		3.25	4.27	4.45	3.15	2.68	3.94
mean	118	3.59	4.34	4.78	3.32	3.16	4.22
s.d.m.		0.209	0.217	0.558	0.156	0.694	0.641

TABLE X

EFFECTS OF SODIUM AZIDE ON THE RESPIRATION OF DCA HYPERTENSIVE RAT KIDNEY SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	240	4.53	4.84	4.74	3.80	4.05	3.99
		4.56	4.44	4.88	3.84	3.60	4.20
	146	3.90	4.73	5.04	3.58	3.75	4.20
		3.80	4.81	5.12	3.50	4.01	4.36
	134	3.39	4.92	5.08	3.13	3.72	4.12
		3.95	4.49	5.10	3.60	3.86	4.29
	178	3.95	4.59	4.76	3.47	3.52	3.85
		4.20	4.02	4.75	3.90	2.64	4.01
	102	4.25	5.10	4.93	4.04	3.86	4.27
		-	5.16	4.96	-	4.27	4.33
	176	3.91	5.67	5.69	3.58	4.33	4.60
		4.12	5.47	6.02	3.85	2.91	4.89
	208	3.51	5.20	5.20	3.47	3.64	4.66
		3.91	5.18	5.18	3.81	3.72	4.30
	154	3.43	4.35	5.60	3.16	3.16	4.12
		4.06	4.70	5.18	2.66	3.00	4.21
	192	3.28	4.38	4.21	2.70	3.30	4.06
		4.24	-	4.21	3.47	-	3.75
	-	4.27	5.80	5.40	3.64	4.20	3.40
		4.31	5.46	-	3.77	3.97	4.56
	190	3.33	4.38	4.88	3.07	3.38	4.19
		3.66	4.44	5.18	3.46	3.38	4.34
mean	172	3.93	4.86	5.05	3.50	3.63	4.21
s.d.m.		0.374	0.477	0.422	0.363	0.445	0.311

TABLE XI

EFFECTS OF HYDRALAZINE ON THE RESPIRATION OF NORMOTENSIVE RAT KIDNEY SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	114	3.00	2.79	4.45	2.77	2.44	3.67
		3.33	3.52	4.65	3.08	2.83	3.84
	102	2.92	3.64	4.29	2.78	3.18	3.60
		3.44	3.73	4.22	3.22	3.12	3.78
	112	3.50	4.37	5.10	3.20	3.46	4.17
		3.68	4.64	4.76	3.18	3.50	4.02
	110	3.02	4.30	4.46	2.62	2.66	3.70
		3.42	3.98	5.05	2.84	2.82	4.10
	122	3.45	4.45	4.80	3.19	3.68	4.20
		3.55	4.33	4.80	3.38	3.64	4.20
	116	3.35	4.20	5.46	3.07	3.25	4.48
		3.82	4.20	4.91	3.50	2.65	4.22
	118	3.14	4.00	4.24	3.10	2.86	3.70
		3.77	3.62	4.14	3.62	2.76	4.14
	118	3.44	4.14	4.66	3.16	2.89	4.51
		4.03	4.48	4.66	3.74	3.24	4.03
mean	114	3.43	4.02	4.67	3.15	3.06	4.02
s.d.m.		0.307	0.495	0.349	0.316	0.373	0.273

TABLE XII

EFFECTS OF HYDRALAZINE ON THE RESPIRATION OF DCA HYPERTENSIVE RAT KIDNEY SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	152	2.64	3.48	3.20	2.34	2.10	2.98
		3.00	2.78	3.59	2.64	2.43	2.65
	118	2.86	3.85	3.35	2.74	2.71	3.03
		2.99	3.20	3.94	2.80	2.30	3.39
	178	2.74	3.12	4.26	2.59	2.39	4.00
		3.09	3.21	4.56	2.80	2.48	3.76
	142	2.57	3.68	3.80	2.45	2.72	3.56
		2.98	3.87	3.72	2.77	2.60	3.41
	176	3.16	4.17	4.28	3.02	3.05	3.52
		3.29	4.06	4.36	3.18	3.29	3.80
	172	2.88	3.76	4.22	2.72	2.37	3.50
		3.30	3.69	4.03	3.15	2.69	3.36
	146	3.23	4.16	4.07	3.01	3.07	3.56
		3.41	4.48	5.16	3.23	3.47	4.16
	134	3.60	4.50	5.06	3.29	3.58	3.70
		3.48	4.17	5.16	3.19	3.72	3.79
	172	2.74	4.10	3.29	2.52	3.12	2.92
		2.80	3.86	3.86	2.52	2.98	3.30
mean	154	3.04	3.85	4.11	2.83	2.84	3.47
s.d.m.		0.290	0.469	0.583	0.288	0.458	0.380

TABLE XIII

SUMMARY OF MEAN QO_2 VALUES OF NORMAL AND HYPERTENSIVE RAT KIDNEY

	Mean Blood Pressure (mm.Hg.)	KRP			KMIII			HM		
		Number of Expts.	0'	60'	Number of Expts.	0'	60'	Number of Expts.	0'	60'
Normal	117	22	3.53	3.08	24	4.32	3.35	22	4.70	4.37
Grollman	183	16	2.82	2.36	18	2.97	2.11	12	3.24	2.96
DCA & Saline	219	16	2.57	2.35	16	2.89	2.14	16	2.95	2.71
DRUG TREATED										
Reserpine N	113	29	3.65	3.28	29	4.23	3.17	25	4.82	4.28
H	171	16	3.53	3.16	15	3.81	2.82	14	4.30	3.86
Sodium Azide N	118	14	3.59	3.32	16	4.34	3.16	13	4.72	4.22
H	172	21	3.93	3.50	21	4.86	3.63	21	5.05	4.21
Hydral- N azine H	114	16	3.43	3.15	16	4.02	3.12	16	4.67	4.02
	154	18	3.04	2.83	18	3.85	2.84	18	4.11	3.47

N - normal animals

H - hypertensive animals

(b) Heart

(i) Reserpine

The samples of heart were used from the same animals from which the kidney samples were obtained. Tables XIV and XV show the QO_2 values of heart removed from normal and hypertensive animals treated with reserpine. Except for a significantly higher QO_2 value in reserpine treated normal heart in KRP, there were no differences in the QO_2 values from the normal untreated rats. This would indicate that reserpine tended to prevent the decreased respiration which occurred in heart removed from untreated DCA hypertensive rats.

(ii) Sodium Azide

The QO_2 values for heart from normal and hypertensive rats treated with sodium azide showed no difference from normal untreated heart except for the normal treated heart in HM flasks which had a significantly higher QO_2 than normal. The QO_2 values for sodium azide treated rats are presented in Tables XVI and XVII.

(iii) Hydralazine

Tables XVIII and XIX show the QO_2 values of normal and DCA implanted rats when hydralazine was administered. No significant difference from the mean of non treated normal animals were shown. Table XX presents the complete summary of the mean QO_2 values for heart.

TABLE XIV

EFFECT OF RESERPINE ON THE RESPIRATION OF NORMOTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	80	1.23	2.23	2.47	0.79	1.07	1.84
		1.29	2.42	-	0.82	1.33	-
	74	1.90	2.36	3.20	1.07	0.80	2.55
		1.78	2.17	3.31	0.98	0.78	2.57
	126	0.81	1.74	3.20	0.78	1.06	2.45
		1.45	2.07	-	1.27	0.84	-
	120	1.51	1.82	2.34	1.00	0.48	1.41
		1.51	2.21	3.00	1.00	1.17	2.03
	140	2.18	-	2.70	1.17	0.70	2.58
		1.93	2.68	3.00	0.90	1.16	2.44
	114	1.23	2.06	3.08	0.84	1.14	2.10
		0.94	1.94	2.87	0.63	0.70	2.50
	132	2.24	2.90	2.94	1.78	1.04	2.68
		2.12	2.64	3.18	1.45	1.22	2.70
	110	1.30	-	1.92	0.80	-	1.54
		-	-	2.33	-	-	1.98
	106	1.10	1.51	3.34	0.78	0.67	2.18
		0.61	1.87	3.55	0.39	0.86	2.36
	108	1.07	1.23	2.43	0.67	0.82	1.89
		0.61	1.92	2.47	0.34	0.73	1.98
	122	1.26	1.53	3.09	1.00	1.21	2.12
		0.66	1.78	2.94	0.46	1.00	2.00
	120	1.01	1.90	3.32	0.79	0.68	1.92
		0.94	1.36	3.36	0.60	0.46	2.17
	110	1.08	2.20	3.30	0.75	0.76	2.00
		0.88	2.00	3.64	0.44	0.64	2.08
	114	0.90	1.70	3.11	0.55	1.14	2.04
		1.42	1.96	3.38	1.15	0.84	2.58
	118	0.85	1.48	2.43	0.70	1.16	1.97
		0.64	1.13	2.25	0.43	0.75	2.00
mean	113	1.26	1.96	2.93	0.84	0.90	2.17
s.d.m.		0.470	0.425	0.437	0.321	0.233	0.324

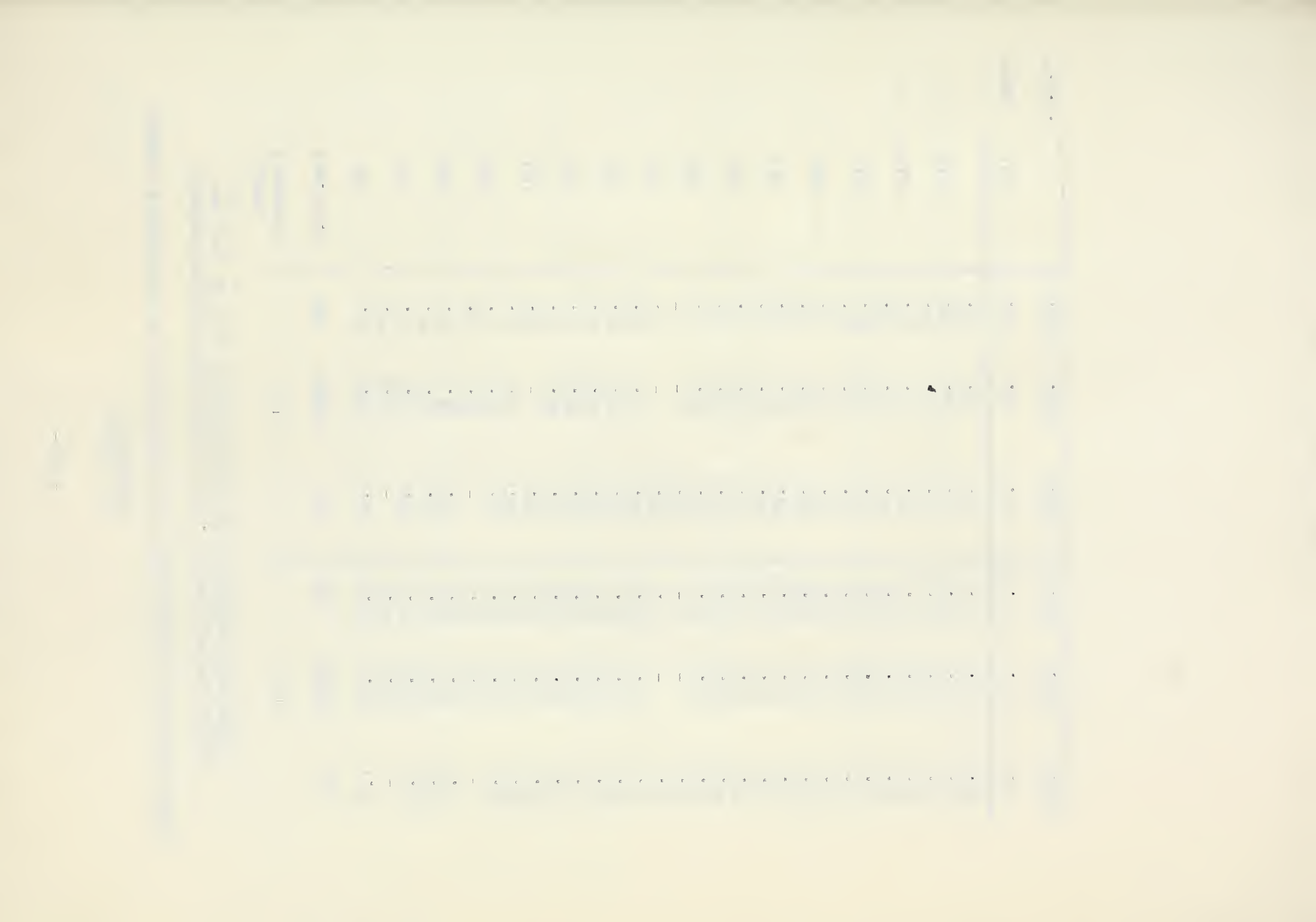


TABLE XV

EFFECTS OF RESERPINE ON THE RESPIRATION OF DCA HYPERTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	160	0.87	1.72	3.20	0.42	0.34	2.16
		1.39	2.38	3.10	1.25	0.95	1.68
	174	0.43	1.50	2.89	0.32	0.50	1.80
		0.99	1.99	2.97	0.87	0.95	2.52
	220	1.18	2.03	2.85	0.93	0.86	2.33
		1.54	2.36	3.23	0.98	1.36	2.37
	112	1.22	1.48	1.82	0.90	0.36	1.80
		0.92	1.48	2.00	0.64	0.66	1.60
	158	1.06	1.61	2.54	0.81	1.12	1.80
		0.95	1.48	2.97	0.80	0.36	1.60
	234	1.13	1.42	3.00	0.91	0.45	1.72
		0.94	1.72	3.04	0.60	0.45	1.81
	198	0.91	1.51	2.04	0.69	0.43	1.39
		0.56	1.40	2.37	0.38	0.55	1.50
	188	0.83	1.34	2.44	0.75	0.35	1.53
		0.55	1.68	2.70	0.50	0.58	1.90
mean	171	0.97	1.69	2.70	0.73	0.64	1.77
s.d.m.		0.287	0.318	0.434	0.241	0.304	0.400

TABLE XVI

EFFECTS OF SODIUM AZIDE ON THE RESPIRATION OF NORMOTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	128	1.37	2.52	2.79	1.14	0.83	2.43
		1.22	2.74	3.15	1.00	1.20	2.74
	122	1.28	2.11	2.66	0.92	0.49	2.04
		0.85	2.11	2.76	0.57	0.49	2.30
	128	0.88	1.96	2.13	0.60	0.55	2.03
		0.98	1.96	2.55	0.71	0.70	2.13
	118	-	2.06	-	-	0.41	-
		-	2.06	2.32	-	0.41	2.06
	114	1.23	1.76	3.10	1.11	0.44	2.36
		1.14	1.76	3.43	0.96	0.51	2.59
	108	1.66	1.60	3.42	1.32	0.55	2.42
		1.24	1.72	3.68	1.02	0.46	2.22
	116	0.70	2.56	2.99	0.31	0.79	2.12
		0.40	2.04	3.21	0.48	1.16	2.32
	110	1.12	1.46	3.27	0.82	0.40	2.10
		0.95	1.37	2.88	0.75	0.36	2.46
mean	118	1.07	1.99	3.02	0.84	0.62	2.29
s.d.m.		0.300	0.371	0.389	0.343	0.217	0.207

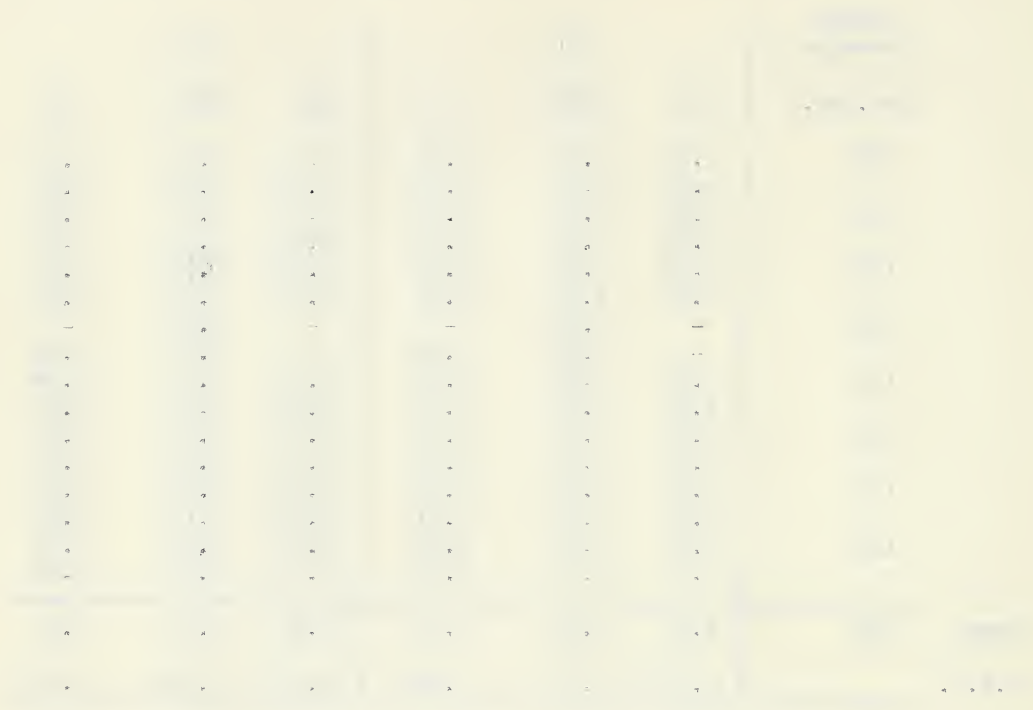


TABLE XVII

EFFECTS OF SODIUM AZIDE ON THE RESPIRATION OF DCA HYPERTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	240	-	0.82	2.92	-	0.51	1.51
		-	0.88	2.97	-	0.55	2.22
	146	1.30	1.76	3.59	0.49	0.49	1.72
		0.87	1.62	3.74	0.45	0.48	2.37
	134	1.77	2.03	2.75	1.60	1.48	2.42
		1.15	1.85	2.75	0.86	0.79	2.42
	178	1.39	2.26	2.62	1.20	0.88	2.16
		1.39	1.90	2.98	1.20	1.25	2.64
	145	1.50	1.70	2.98	1.30	0.79	2.46
		0.69	-	2.83	0.52	-	2.26
	176	1.91	2.24	3.14	1.60	1.84	2.82
		1.20	1.63	2.78	0.80	1.03	2.56
	208	0.65	1.78	2.46	0.48	1.22	1.94
		1.18	1.73	2.93	1.10	0.68	2.43
	154	0.65	1.18	3.19	0.42	0.54	1.88
		0.59	-	3.37	0.36	-	1.95
	192	1.64	2.00	2.80	1.35	1.28	2.54
		1.02	1.92	2.76	0.76	1.24	2.76
	-	1.23	1.49	2.09	0.90	0.80	1.60
		0.58	1.60	2.70	0.38	1.17	1.40
	190	0.71	1.12	2.08	0.60	0.44	1.60
		0.71	1.60	2.48	0.60	0.80	1.40
mean	172	1.11	1.66	2.86	0.85	0.91	2.14
s.d.m.		0.404	0.389	0.395	0.400	0.377	0.439

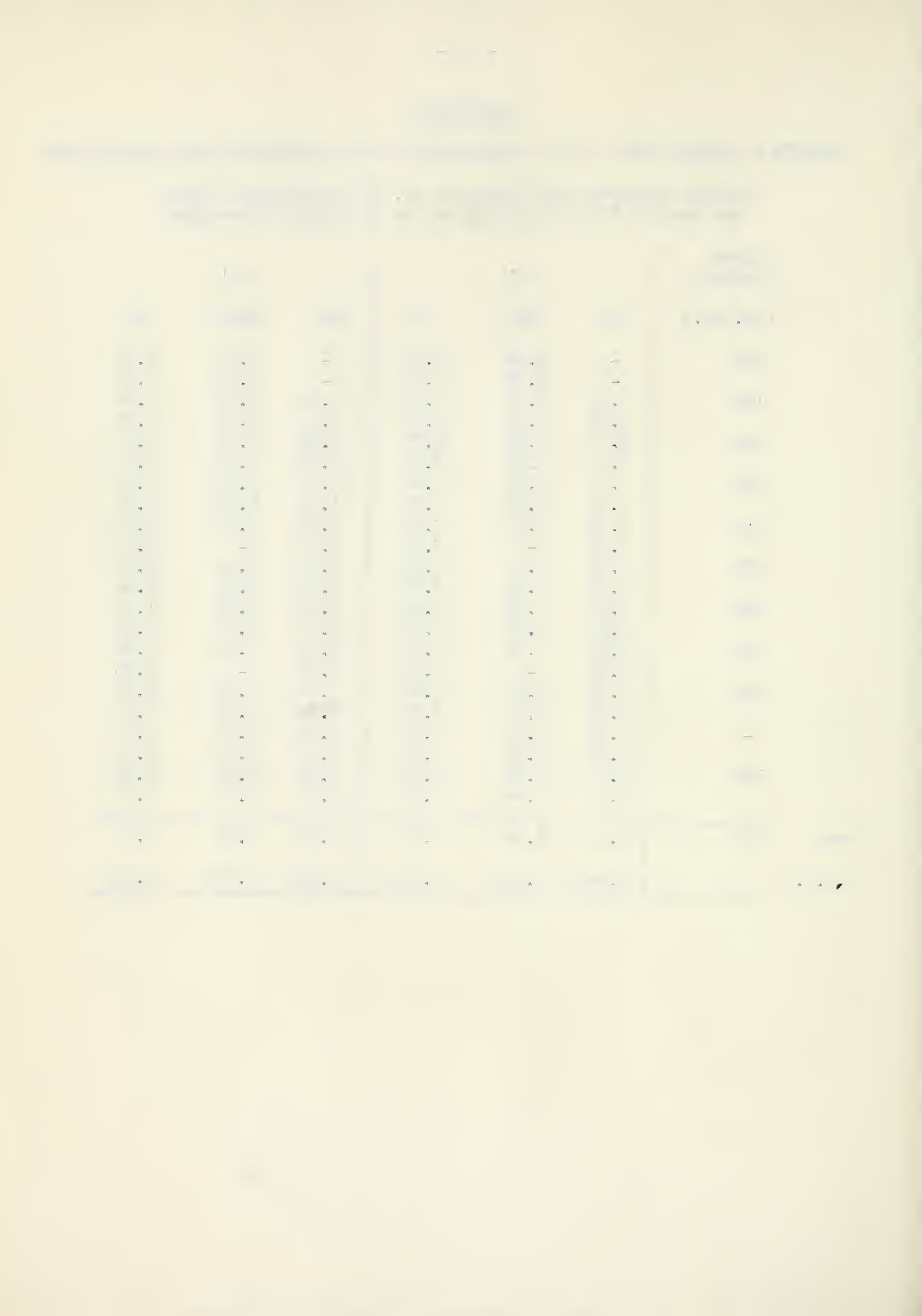


TABLE XVIII

EFFECTS OF HYDRALAZINE ON THE RESPIRATION OF NORMOTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	114	1.58	2.28	2.64	1.02	0.60	2.54
		1.25	2.28	2.99	0.82	0.60	2.70
	102	1.16	2.20	2.37	1.02	1.08	2.23
		0.85	1.86	2.95	0.72	1.02	2.58
	112	1.41	2.26	3.38	1.09	0.62	1.92
		0.86	2.42	3.60	0.72	0.55	2.33
	110	1.68	2.00	-	0.86	0.62	1.93
		1.44	1.98	-	0.64	0.39	1.93
	122	1.52	1.22	3.00	1.20	0.32	1.94
		1.16	1.50	2.46	0.94	0.32	1.53
	116	1.45	1.50	3.18	1.29	0.46	2.03
		0.81	1.68	3.16	0.72	0.50	2.15
	118	1.35	2.45	2.35	1.14	1.28	1.88
		0.76	1.56	2.90	0.65	0.84	2.37
	118	0.47	2.22	2.97	0.11	0.66	1.06
		0.44	1.87	2.97	0.23	0.56	1.40
mean	114	1.14	1.96	2.92	0.82	0.65	2.03
s.d.m.		0.379	0.363	0.352	0.316	0.265	0.373

TABLE XIX

EFFECTS OF HYDRALAZINE ON THE RESPIRATION OF DCA HYPERTENSIVE RAT HEART SLICES

Figures represent QO_2 values in ml. O_2 per gram wet weight per hour at time of setting and at 60 minutes thereafter

	Blood Pressure	0'			60'		
	(mm. Hg.)	KRP	KMIII	HM	KRP	KMIII	HM
	152	0.96	1.34	2.57	0.65	0.44	1.13
		0.40	1.55	3.09	0.30	0.34	1.08
	118	0.79	2.00	2.25	0.52	0.62	1.60
		0.52	1.45	3.02	0.28	0.34	0.80
	178	1.40	2.02	2.82	0.98	0.70	2.35
		1.01	2.10	2.57	0.73	0.67	2.18
	142	1.48	1.03	2.42	0.86	0.40	1.26
		0.66	1.57	2.78	0.57	0.42	1.46
	176	0.93	2.22	2.55	0.65	0.60	1.64
		0.93	1.94	2.33	0.65	0.60	1.76
	172	0.78	1.79	2.27	0.37	0.80	1.00
		-	1.14	2.48	-	0.44	1.38
	146	0.70	1.94	2.64	0.43	0.81	1.04
		0.77	1.80	2.52	0.65	0.47	1.20
	134	0.82	2.00	2.75	0.54	0.50	1.27
		0.48	1.90	2.87	0.32	0.77	1.10
	172	1.62	1.49	2.36	0.80	1.01	1.86
		0.95	1.98	3.04	0.64	0.69	1.90
mean	154	0.89	1.74	2.63	0.58	0.59	1.45
s.d.m.		0.327	0.332	0.257	0.080	0.572	0.418

TABLE XX

SUMMARY OF MEAN QO_2 VALUES OF NORMAL AND HYPERTENSIVE RAT HEART

	Mean Blood Pressure (mm.Hg.)	KRP			KMIII			HM		
		Number of Expts.	0'	60'	Number of Expts.	0'	60'	Number of Expts.	0'	60'
Normal	117	26	1.00	0.62	33	1.79	0.63	32	2.73	1.83
Grollman	183	16	0.71	0.47	18	1.60	0.81	16	2.28	1.54
DCA & Saline	219	16	0.78	0.55	16	1.73	0.71	15	2.36	1.53
DRUG TREATED										
Reserpine N	113	29	1.26	0.84	27	1.96	0.90	28	2.93	2.17
H	171	16	0.97	0.73	16	1.69	0.64	16	2.70	1.77
Sodium Azide N	118	14	1.07	0.84	16	1.99	0.62	15	3.02	2.29
H	172	20	1.11	0.85	20	1.66	0.91	22	2.86	2.14
Hydral- N	114	16	1.14	0.82	16	1.96	0.65	14	2.92	2.03
azine H	154	17	0.89	0.58	18	1.74	0.59	18	2.63	1.45

N - normal animals

H- hypertensive animals

5. Comparison of Drug Treatment on the QO_2 Values of Heart and Kidney

Since the level of response at zero time is believed to represent most accurately the situation in the animal, chief emphasis should be placed on this figure when evaluating the in vivo effect. A summary of the response in relation to the normal QO_2 values for heart and kidney is presented in Table XXI and Figure IV. Examination of this table and figure reveals that all drugs tested prevented the significant decrease in the QO_2 values of the heart in DCA hypertensive animals in KRP and HM flasks. It is also observed that the decreased respiration of the DCA hypertensive kidney was prevented by reserpine. Sodium azide treated DCA hypertensive rats showed a significantly valid increased oxygen consumption while hypertensive rats treated with hydralazine showed a significantly decreased QO_2 value in all flasks. It should be noted however that the amount of decrease of QO_2 values in the hydralazine treated rats was not as great as that of DCA implanted rats which were not receiving the drug.

TABLE XXI

SUMMARY OF MEAN QO_2 VALUES OF HEART AND KIDNEY EXPRESSED AS A PERCENTAGE OF NORMAL MEAN QO_2 AT ZERO TIME

	KIDNEY			HEART		
	KRP	KMIII	HM	KRP	KMIII	HM
Normal	3.53	4.32	4.70	1.00	1.79	2.73
Grollman	S 79.9	S 69.7	S 68.9	S 71.0	89.4	S 83.5
DCA & Saline	S 72.8	S 66.9	S 62.8	S 78.0	96.6	S 86.4
DRUG TREATED						
Reserpine N	103.4	97.9	102.6	S 126.0	109.5	107.3
H	100.0	S 88.2	91.5	97.0	94.4	98.9
Sodium Azide N	101.7	100.5	101.7	107.0	111.1	S 110.6
H	S 111.3	S 112.5	S 107.4	111.0	92.7	104.8
Hydral-azine N	97.2	93.1	99.4	114.0	109.5	107.0
H	S 86.1	S 89.1	S 87.4	89.0	97.2	96.3

N - normal animals

H - hypertensive animals

S - a significant difference from normal

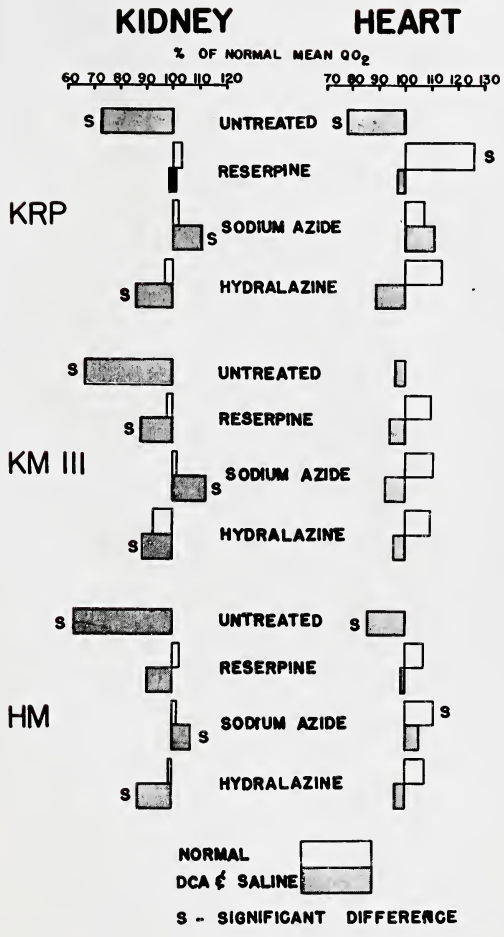


FIGURE IV

Effects of Drug Treatment on the Respiration of Heart and Kidney from Normotensive and DCA Hypertensive Rats Expressed as a Percent of Mean Normal QO_2 Values at Zero Time

VII. DISCUSSION

1. Production of Hypertensive Rats

(a) Choline-Free Diet

The method of using a short period of choline deficiency in the early life of rats with or without a sodium chloride solution as a drinking water thereafter failed to produce any substantial increase in the blood pressure. Best and Hartroft (27), Handler and Bernheim (31) and Moses, Longebaugh and George (30) did obtain hypertension in one third to one half of the rats surviving the diet. Contrary to these reports Sobin and Landis (28) could obtain no hypertensive rats and Knudson and Harris (32) found only moderate success with this method. The latter workers found that of the rats surviving a choline deficient diet only 35% had a blood pressure above 124 mm. Hg and these had a mean pressure of only 138 mm. Hg. Sobin and Landis concluded that "the striking renal lesions of acute choline deficiency do not belong to the renal abnormalities which frequently produce hypertension". Although this method may be of interest in nutritional studies it would appear to have little utility in the production of experimental hypertension in rats.

(b) Grollman

The Grollman method of producing hypertensive rats is said to result in a hypertensive condition which resembles clinical hypertension in its pathological and hemodynamic features (33). However, for determining the long term protective effects of antihypertensive agents the method has several disadvantages. All animals operated on do not develop a

persistant hypertension. Murray and Nelson (26) reported that 73.5% of the male rats operated upon developed a consistantly high blood pressure. Grizzle (13) obtained a very low percentage of hypertensive animals using this technique.

The results of this investigation showed that only eleven of twenty rats or 55% of animals subjected to the Grollman operation developed hypertension. It must be noted that in this work ligatures were placed around both kidneys whereas the other workers removed one kidney and tied a ligature around the remaining one. Although the removal of one kidney is said to result in a faster development of hypertension both kidneys were required to ensure sufficient tissue for the tissue respiration studies. The rate at which animals developed hypertension by this method varied so that the animals were not sacrificed after a definite time interval but rather when a persistantly high level of blood pressure had resulted. The most serious disadvantage of the Grollman type of hypertension was that it resulted in seriously damaged kidneys. It was difficult to obtain good slices from the kidneys of Grollman hypertensive animals.

(c) DCA

DCA hypertension is said to more closely resemble human essential hypertension than any other type of experimental hypertension (41). One hundred percent of the rats implanted with DCA pellets and given saline solution to drink developed a definite rise in blood pressure after three weeks. Gaunt et al (42) reported that the mean survival time of rats so treated was seventy one days but it was found that after a period of forty to fifty days serious losses of rats occurred and it was decided to sacrifice the animals at this time. At the time of the respiration

studies all animals had a persistant hypertension which was considerably higher than that obtained by the Grollman method.

Because the DCA method produced hypertensive rats more consistently and more quickly than the Grollman method and because the kidney was in a more satisfactory condition to obtain good kidney slices it was decided that this method would be the most suitable to use to study the effects of the antihypertensive agents.

2. General Effects of the Antihypertensive Agents

DCA implanted rats which were treated with the three antihypertensive agents had a lower blood pressure after the waiting period than those DCA implanted rats which were not treated. The antihypertensive drugs had little effect on the blood pressure of the normal animals. Reserpine and hydralazine lowered the normal pressures slightly but sodium azide had no effect on the normal blood pressure.

Grizzle (13) reported that a 0.025% solution of sodium azide as the drinking water did not lower the blood pressure of normal or Grollman hypertensive rats. However, Grizzle failed to develop a definite hypertension in the rats. He also reported that the long term administration of sodium azide was well tolerated and that no toxic symptoms developed. He did report that some animals lost weight during the forty day treatment.

This investigation showed that the administration of sodium azide over the period studied resulted in a lower mean blood pressure in the DCA hypertensive rats than those untreated. The total effects of the drug, however, did not appear to be entirely beneficial. At the end of the waiting period those rats which had survived were very weak and had failed to gain weight. Only 78.6% of the sodium azide treated hypertensive rats survived this period as compared with 81.8% treated with reserpine and

100% with hydralazine. Only 61.6% of the untreated DCA implanted rats survived the waiting period so that all drugs tested did have some beneficial effects.

The hypertensive animals treated with reserpine and hydralazine appeared much stronger and healthier than those treated with sodium azide. Although both reserpine and hydralazine produced a lower mean blood pressure than untreated DCA implanted rats, hydralazine was superior in this respect. The results of the blood pressure and percent survival agree quite well with the reports of Gaunt et al (42) and Gross et al (46). These workers found that reserpine and hydralazine reduced the blood pressure of "metacortoid" hypertension with reserpine being less effective than hydralazine.

Sodium azide and reserpine reduced the saline intake of the DCA hypertensive rats but hydralazine had no effect in this respect. Similar results were also reported by Gaunt et al (42). Both sodium azide and reserpine may produce their protective effect in part by reducing the salt intake but hydralazine, which afforded the best protection and lowered the blood pressure more than the other two drugs, must produce its beneficial effects by some other, and as yet unknown, method.

3. Tissue Respiration

(a) General Discussion

A discussion of the results of the tissue respiration should be prefaced by some comments on the procedure proposed by Huston and Martin. The in vitro evaluation of the pharmacological action of drugs at the cellular level after administration of the drug in vivo is complicated in standard Warburg methods by such factors as modification of the drugs

and tissue metabolites by the liquid suspension medium. Cellular effects demonstrated by the addition of drugs *in vitro* may or may not represent the response in the intact animal, particularly in view of possible differential tissue distribution and sensitivity of the drug. Huston and Martin showed that tissue respiration can be measured with the tissues suspended in a gaseous phase of oxygen on fibre glass mats. This technique avoids variations due to different liquid media and permits quantitative assessment *in vitro* of the tissue effects of the drugs administered to the intact animal.

Rodnight and McIlwain (74) compared rates of respiration of brain, kidney, diaphragm and liver without added media and in olive oil, light paraffin and silicone fluid. In each case respiration rates were initially higher than those observed in saline which were run at the same time. They found that unless glucose was added the rate of respiration fell quite rapidly in brain.

Drabkin and Marsh (77) using a moist chamber respirometer on a principle similar to that of the Huston Martin technique found that they could incubate tissue slices for as long as ten hours without appreciable diminution of the rates of oxygen uptake. They found that tissues remained viable two to three times longer than in conventional Warburg technique.

Some advantages to the technique of administering the drug to the animal and examining the tissues in oxygen would appear to be:-

(a) the drug has been administered *in vivo* and the distribution and response has been governed by the intact animal; adding the drug from the side arm *in vitro* presents a completely artificial situation;

(b) the drug has not been diluted or extracted from the tissues by a liquid medium;

(c) variable influences due to ions or metabolites in the medium are avoided.

Disadvantages or limitations of the procedure may be summarized as follows:-

(a) once the tissue is placed on the mat further drugs and/or metabolites cannot be added to it. The technique therefore does not lend itself to an examination of substrate phenomena;

(b) the tissue cannot act as its own control as is the case when the drug is added from a side arm. It is necessary to run control series of non treated animals;

(c) a not too serious disadvantage and one which is inherent in all tissue respiration studies is that once the tissue is removed from the body it progressively departs from physiological normalcy. Many factors are involved not all of which are known. Some of the more obvious factors are loss of hormone and nervous control, limitation of supply of metabolites and ions and accumulation of metabolic end products. However, since the primary interest is the effect of the drug on the tissue at the time it is removed from the body, that is the in vivo effect, this disadvantage is not serious.

(d) a possible disadvantage is that the tissue in contact with oxygen and not supplied with nutrient may burn itself up. This situation would be indicated by a more rapid fall in slope of the graph and at the end of an hour the respiration rate might be expected to be below that of the tissues in fluid. However it was found that the tissues on the mats had the least diminution of oxygen consumption (77)(79).

The above disadvantages are minimized by the procedure of extrapolation to zero time. Rate of respiration has been reduced in the cold during the preliminary manipulations and returns to a maximum at the conclusion of equilibration which is the point of extrapolation. This figure, so obtained, would appear to most closely approximate the in vivo situation.

Even though the zero time results are of more significance as they represent what the situation is in the intact animal, examination of the sixty minute values show that both the heart and kidney tissues in the HM flasks remain nearer the initial zero time QO_2 value than the tissues in the two media. The HM flasks appear to be well adapted for heart respiration. At sixty minutes the normal heart was respiring at 62% of its zero time value in KRP, 35% in KMIII and 67% in the HM flasks. Kidney slices respired at a much more constant rate but here too the rate at sixty minutes was higher in the HM flasks than in the two liquid media. In HM flasks the kidney tissues respired at 93% of the zero time rate, 77.5% in KMIII and 87% for KRP. These sixty minute values show that the higher zero time QO_2 values obtained in the HM flasks are real and not due to extrapolation. Because of the low sixty minute QO_2 values in KMIII the extrapolated zero time may be higher than actually is the case.

(b) Tissue Respiration of Normal and Hypertensive Tissues

The confusion which exists in the literature concerning the respiration of hypertensive tissue is due in part to the variations of techniques used. The administration of the antihypertensive drugs to the intact animal is the only procedure which could be used in a study of this kind. The disadvantages of using regular Warburg technique have been discussed and it would appear that the technique proposed by Huston and Martin would be a useful method for investigating the effects of certain antihypertensive drugs on the tissues of normal or hypertensive animals.

Previous work dealing with the oxygen consumption of hypertensive tissues was concerned only with tissues removed from animals which were made hypertensive by clamping the renal artery. It seems to be agreed that the ischemic kidney in this type of hypertension does have a lower oxygen consumption and enzymatic activity than normal kidney (56 to 61). It was shown (Figure III) that the kidneys of both the Grollman and DCA hypertensive animals had a significantly lower oxygen consumption than the kidney tissue of normal animals. It would appear that irregardless of the type of hypertension the kidney has a lower rate of respiration than the kidney of normal animals.

Ruskin et al (60) are the only workers who have reported the effects of hypertension on the respiration of the heart. They found that there was a moderate decrease in the succinic dehydrogenase activity in the heart of the Goldblatt type hypertensive animal. In our work oxygen consumption of heart from both Grollman and DCA hypertensive rats showed a significant decrease in KRP and HM flasks (Figure III). A decreased mean QO_2 value was observed in KMIII but this decrease was not large enough to be statistically valid.

(c) The Effect of Drugs on the QO_2 Values of Normal and Hypertensive Tissue

(i) Normal Kidney

There were no significant changes in the QO_2 values of normal kidney removed from animals treated with sodium azide, reserpine or hydralazine.

(ii) Hypertensive Kidney

(A) Reserpine

Kidney removed from DCA hypertensive rats which did not receive drug treatment showed a decreased rate of oxygen consumption in all flasks. The QO_2 values of hypertensive rats treated with reserpine showed no significant difference from that of normal QO_2 values in KRP and HM flasks. Kidney tissue in KMIII presented a significantly decreased QO_2 value but this value was not as reduced as that of the untreated hypertensive kidney slices in that medium. It would appear that as well as reducing the blood pressure, reserpine protected the kidney from the biochemical change which must take place in that organ during the development of DCA hypertension.

(B) Hydralazine

Although hydralazine lowered the blood pressure and increased the percentage of survival better than reserpine, it did not effect the tissue respiration of the hypertensive kidney as dramatically as reserpine. The mean QO_2 values of kidney in DCA implanted rats were significantly lower than normal in all flasks. The observed decrease of QO_2 values in the hydralazine treated animals was not as great as that observed in the untreated animals and thus hydralazine afforded some protection to the hypertensive kidney. Since hydralazine is said to increase the renal blood flow it might be through this factor that hydralazine affords some protection to the DCA hypertensive kidney.

(C) Sodium Azide

The kidneys of rats made hypertensive by DCA and treated with sodium azide showed a significant increased QO_2 value in all flasks.

Since it was first reported by Keilin (12), sodium azide has been regarded as a metabolic inhibitor. For this reason it might be expected that administration of the drug would result in a decreased oxygen consumption. Since the hypertensive animals treated with this drug were very ill at the time of the tissue respiration studies it may be true that a stress reaction or toxic condition produced the increased oxygen uptake. Other than this and the fact that most previous work on cell metabolism with the drug has been done by tipping a solution of the drug into the Warburg flask from a side arm no explanation can be given about the increased oxygen consumption brought about by the administration of sodium azide.

(iii) Heart

The kidney and not the heart is thought to be actively engaged in some aspect of the etiology of hypertension and thus little work has been concerned with tissue respiration of the heart from hypertensive animals. Since the drugs studied lowered the blood pressure it is felt that less work would be done by the heart and therefore less damage to its tissues would occur. Other than the fact that the QO_2 values of normal rat heart were significantly increased in KRP when treated with reserpine and in HM flasks when treated with sodium azide there were no significant changes from the QO_2 of normal untreated animals. No adequate explanation to account for the two significant increases presents itself.

It was found that when antihypertensive agents were administered to the DCA implanted rats the respiration of the heart was not significantly decreased as in the case of the untreated hypertensive rats.

(d) Discussion of Huston-Martin Technique

As well as testing the effects of antihypertensive agents on tissue respiration it was the aim of this research to examine the HM

technique as a tool for pharmacological investigations. As a criterion for the results obtained in HM flasks, two solutions were used, one rich in substrate (KMIII) and one a simple saline solution. Any inadequacy of the Huston-Martin procedure should have been apparent.

It would appear from our results that the technique of determining tissue respiration in oxygen is a useful tool for pharmacologic studies. The level of response of normal animals was higher in the HM flasks than either of the liquid media and agrees within experimental limits with those reported by Huston and Martin (79)(80). These authors reported a greater sensitivity for the method in examining the effects of dinitrophenol and sodium arsenite on tissue respiration. It would seem that a greater sensitivity was noted in the HM flasks than the two liquid media in the case of the kidney respiration of the hypertensive rats. In studies of heart respiration the Huston-Martin technique had a definite advantage since the readings with the KRP solution were small and inconsistent and those with the KMIII solution diminished very quickly. In both KRP and KMIII the rate of respiration of heart and kidney slices declined more rapidly than in the HM flasks.

In order to study the effects of the antihypertensive drugs on both the blood pressure and tissue respiration it is obvious that the drug cannot be tipped in from the side arm but must be administered to the animal prior to the tissue respiration studies. Thus it is felt that the Huston-Martin technique is well adapted to this type of research problem.

The findings of this work would seem to indicate that the Huston-Martin technique has several advantages over the conventional Warburg

technique in pharmacologic studies. Any inadequacies of the Huston-Martin method are largely those inherent in any procedure where the tissues are removed from the animal and studied in vitro.

VIII. SUMMARY AND CONCLUSIONS

The aim of this research was to determine the tissue respiration of heart and kidney from normal and hypertensive rats and secondly to determine the effects on this respiration of certain antihypertensive agents.

Three procedures were used to develop hypertension in rats:- choline-free diet, Grollman operation and the administration of DCA and saline. Of the techniques used to produce the experimental hypertension the DCA method was found to be the best for our purposes and the effects of the antihypertensive agents were determined on rats made hypertensive by this method. A satisfactory high level of blood pressure was obtained with the Grollman method but not with the choline free diet.

Reserpine, sodium azide and hydralazine caused a reduction in the mean blood pressure of DCA implanted rats but failed to reduce the pressure to the normal value. Hydralazine appeared to be the most beneficial of the antihypertensive agents studied as it produced the lowest mean blood pressure and the greatest percent of survival. Sodium azide lowered the blood pressure but appeared to produce toxic effects on the rats treated. The antihypertensive agents had no effect on the blood pressure of the normal animals.

The heart and kidney tissues removed from the hypertensive rats had a lower rate of oxygen consumption than the normal tissues, irregardless of the type of hypertension produced. It would appear from our results and those of previous workers that the respiration of hypertensive kidney and heart tissue is lower than the corresponding tissues in normotensive animals.

The administration of the antihypertensive drugs to normal animals had no effect upon the tissue respiration of the kidney or heart.

The effects of the drugs on the DCA hypertensive tissues were as follows:

(a) Reserpine

When reserpine was administered to DCA hypertensive rats it caused the respiration of the kidney to return to normal in KRP and HM flasks and reduced the depression in KMIII. Reserpine brought about a return to normal respiration in the hypertensive heart in all flasks.

(b) Sodium azide

When sodium azide was administered to DCA hypertensive rats there was an increase in the respiration of kidney slices in all flasks. In heart, sodium azide brought about a return to normal respiration.

(c) Hydralazine

The tissue respiration of DCA hypertensive kidney was below that of normal in all flasks when the rat was treated with hydralazine but this reduction was not as great as that in the untreated DCA hypertensive kidney. The respiration of heart removed from hypertensive rats which were treated with hydralazine was at a normal rate.

The decreased respiration observed in the heart of untreated DCA hypertensive rats was returned towards normal when the animals were treated with the antihypertensive drugs. Both reserpine and hydralazine tended to return towards normal the decreased kidney respiration which was observed in non treated DCA hypertensive rats; reserpine was the most effective in this respect. Sodium azide caused an increase in the kidney

respiration of the hypertensive rats. This phenomenon was believed to be a reaction to some toxic effect produced by the drug.

The significance of the findings and the utility of the Huston-Martin technique are discussed.

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